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# STUDIES ON SOIL PHYSICS.

## PART I.—THE FLOW OF AIR AND WATER THROUGH SOILS.

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### *Introduction.*

§ 1.  In connexion with a research on the composition of drainage waters from soils, which is now being undertaken at this laboratory, it was found advisable to have some definite knowledge of the permeability of the soils to water.

The mechanical analysis of soils, as ordinarily carried out, determines the percentages of the various sized particles present; but this determination in the majority of cases gives only indirect and approximate information as to its properties.  For example, we know, in a general way, that a soil containing a large percentage of clay will be only slightly permeable to air or water, and conversely, if it be composed mainly of coarse particles, it will usually be open and easily drained; but the presence or absence of humus material may have an altogether indeterminable effect on these properties if we have merely the usual mechanical and chemical analyses to guide us.

In any case such knowledge is at best only qualitative, whereas the information actually required in the above instances—indeed in any investigation dealing with drainage, irrigation or loss by evaporation—should quantitatively express the relations of the soil to the movements of air and water through it.

These relations are much less obscure if we direct our attention to the number and dimensions of the spaces between the particles rather than to the sizes of the particles themselves.

It will be shewn that the information required can be supplied for a given soil by a knowledge of three quantities which may be regarded, and recorded, as specific constants of the soil.

## § 2.   *Definitions of Soil Constants.*

(*a*)   The *Specific Pore* or *Interstitial Space*, designated by *S*, may be defined as the free space per unit volume of soil.

(*b*)   The *Permeability* to *Water*, designated by $P_w$, may be measured by that volume of water which will pass per second, through a soil column of unit length and of unit area of cross-section when under one centimetre head of water pressure.   The *Permeability* to *Air* may be similarly defined and is designated by $P_a$.

(*c*)   The *Capillarity Coefficient*, designated by *K*, may be defined as the tension due to capillary forces per unit area of cross-section of the pore-spaces which tends to draw the water from the saturated to the dry region of the soil.

§ 3.   King has indeed suggested the direct measurement of the permeability but he uses the data so obtained to calculate the average size of the soil particles, without insisting on the intrinsic value of the permeability constant itself.

This paper describes our attempts to construct a scientific basis for these three fundamental constants and includes an account of the methods adopted for their measurement and the results obtained with three types of soil.

§ 4.   These constants are constants for a particular soil in a particular condition ; they are however all interdependent, and are liable to considerable alteration when the soil is disturbed as by the ordinary farm operations of ploughing, rolling, etc., or by the ordinary process of taking the sample.   Alterations are also caused by variations in the water content.

Obviously, therefore, the measurements should be made on the soil *in situ*, although our preliminary experiments have been made on "disturbed" samples.

The perfection of a method of soil sampling which will not disarrange the original structure, etc., is greatly to be desired, as it would render possible the comparison of the relative efficiency of various methods of cultivation—to mention one of many applications.   An instrument has indeed been devised for this purpose by Stevenson[1], but the present writers have as yet had no opportunity of testing its suitability for their purpose.

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[1] Iowa Agric. Expt. Station, U.S.A., *Bul.* 94, 1908.

*Theoretical Development and Discussion of the Soil Constants.*

§ 5.  (a)  *The Specific Pore Space.*—The *accurate* calculation or measurement of this factor is a far from satisfactory problem when complicated by the ordinary field conditions, and both the permeability and the capillarity of the soil shew considerable variations for a small difference in its value.

In the experiments (to be described in a further paper) with practically uniform spherical glass beads of 0·25 to 1·0 mm. diameter, the pore space varied from 0·40, when the beads were simply poured into the containing vessel, to 0·35, when they were compacted as far as was possible by rolling and tapping.  In the latter state the permeability was found to have been reduced to about 60 per cent. of its original value.

For soils, the specific pore or interstitial space—*i.e.* the free space per unit volume of soil—may be considered either as the actual space available for the interstitial air $(S_a)$ or else as the total space not occupied by the solid matter of the soil $(S_w)$.  The usually slight difference between these two, in moderately dry laboratory samples, is of course due to the moisture present covering each grain of soil with a thin film of water and cutting down the free space available for air, and

$$S_w = S_a + \theta,$$

where $\theta$ is the volume of water present per c.c. of soil.

In a wet or waterlogged soil $S_a$ may be only a small fraction of $S_w$.

In this research the specific pore-space, unless otherwise indicated, has been taken to mean $S_a$.

§ 6.  (b)  *The Permeability to Air and Water.*—We may regard a porous soil as composed of a bundle of capillary tubes, irregular in area, length, direction and shape, but sufficiently minute to reduce the velocity of flow of air or water, under normal conditions, to velocities which conform to Poiseuille's capillary tube law :

$$v = \frac{\pi}{8} \cdot \frac{ghst}{\eta} \cdot \frac{r^4}{l} \quad \dots\dots\dots\dots\dots\dots(1),$$

where        $v =$ volume of liquid passing in time $t$;

and          $s =$ density of liquid;

             $r =$ radius of capillary ;

             $\eta =$ viscosity of liquid ;

$l$ = length of capillary;

$g$ = gravitational constant;

$h$ = head of liquid pressure.

But for a soil the capillaries must be treated statistically and $r^4$ replaced by $\Sigma r^4$ to denote the sum of the fourth powers of the efficient average radii of the capillaries in the area of cross-section $A$ under consideration.
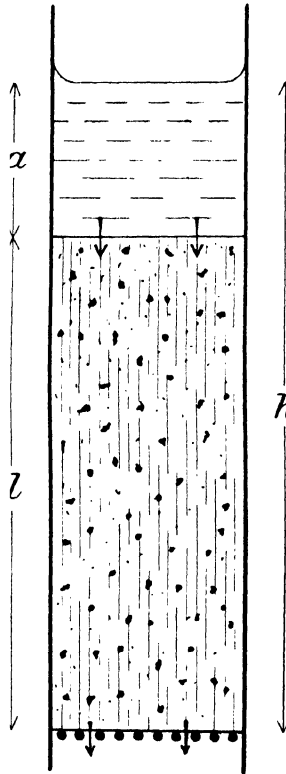


Fɪɢ. 1.

If we consider a vertical tube of soil (Fig. 1), of length $l$, through which water is flowing under the influence of a head of pressure $a + l = h$, and escaping freely at the lower end; then

$$\frac{v}{t} = \frac{\pi}{8\eta} \cdot \frac{ghs}{l} \cdot \Sigma r^4 \quad \dots\dots\dots\dots\dots\dots(2),$$

but $v = Ax$, where $x =$ the distance that any given cross-section of water (of area $A$) has moved in the time $t$; therefore,

$$\frac{x}{t} = \frac{\pi}{8\eta} \cdot \frac{ghs}{l} \cdot \frac{\Sigma r^4}{A} \quad \dots\dots\dots\dots\dots\dots\dots(3)$$

$$= c \cdot \frac{h}{l} \cdot p,$$

where $c = \frac{\pi gs}{8\eta}$ and $p = \frac{\Sigma r^4}{A}$ (the absolute permeability of the soil).

Then
$$cp = \frac{x}{t} \cdot \frac{l}{h} = P_w \quad \dots\dots\dots\dots\dots\dots(4)$$

or
$$P_w = \frac{v}{t} \cdot \frac{l}{Ah} \quad \dots\dots\dots\dots\dots\dots(5).$$

§ 7. $P_w$ is the practical permeability constant of the soil for water, and is equal to the volume of water passing, in unit time, through a soil column of unit area of cross-section under a head of water pressure equal to the length of the column. It may also be expressed as "the linear rate at which water will sink through an area of soil, saturated and just covered with the water."

Whereas $p$, the absolute permeability, is independent of the temperature and depends only on the structure of the soil, $P$ is inversely proportional to the viscosity of the permeating fluid and therefore, when water is employed, varies some two or three per cent. per degree centigrade.

§ 8. In measuring the permeability to air, $P_a$, Meyer has shewn that the working pressure $ghs$ in equation (2) must be replaced by $q_1 - q_2$, where $q_1$, $q_2$ are the pressures (in absolute units) of air at the two ends of the column, or more correctly by $\dfrac{q_1{}^2 - q_2{}^2}{q_0}$, where $q_0$ is the pressure under which the volume $v$ of the air is measured.

Various experimenters, including Darcy (1856), Hazen (1890), King and Slichter (1899), Bell and Cameron (1906) and Leather (1908) have made measurements on the permeability of soils to either air or water and have found that, with certain limitations to be discussed further, both the Poiseuille and Meyer-Poiseuille formulae hold good.

§ 9. From equation (3) $\eta_w P_w$ should be equal to $\eta_a P_a$; or $p$, the absolute permeability, should be independent of the fluid (water or air) experimented with unless the presence of the water is sufficient to modify the capillary passages of the soil.

A critical examination of the results obtained by King[1] and of the measurements to be here described, shew that this modification may take place in two ways: (i) by the moisture in the soil restricting the area of the capillaries through which the air is passing, and hence causing $S_a$ to be less than $S_w$; and (ii) by the humus, clay or other colloidal matter of the soil absorbing water when the soil is wet. The consequent swelling may restrict or even completely close the capillaries in this case.

These considerations again emphasise the advisability of determining the permeability to air and water of the soil *in situ*, without disturbing either its structure or its moisture content.

§ 10. (c) *The Capillary Coefficient.*—Thus far we have considered the permeability of soils to water when they are already saturated, but under ordinary conditions soils are usually less moist than this and may sometimes be almost dry.

Capillary forces must therefore be taken into account, for they assist the hydrostatic pressure when the water is passing downwards and can, in the dry season, even cause moisture to rise to the surface against the force of gravity. The papers of Lyman Briggs, Buckingham and Cameron[2] and of Leather[3] are of great interest in this connexion.

To investigate the nature of the capillary constant we will, as before, consider a column of soil uniformly packed in a tube through which water is percolating from one end.

§ 11. There are three possible cases for consideration, according as the water is travelling vertically downwards or upwards, or horizontally sideways.

1. Of these three possibilities take first the case in which the movement is vertically downwards.

Then the impelling force at any instant will no longer be $ghs$ as in equation (2) but $gs(h + K)$ where $K$ is a constant of the soil depending on the capillary forces acting on the moving boundary of the water.

As the water only occupies the pore-space, $S$, of the soil it follows that at any stage the velocity of the water front will be given by the equation

$$\frac{dv}{dt} = A\,\frac{dl}{dt}\,S \quad \dots\dots\dots\dots\dots\dots\dots\dots(6),$$

[1] *XVth Ann. Rep. Wisc. Agr. Expt. Station*, p. 123.

[2] U.S.A. Bureau of Soils, *Buls.* 10, 19, 30, 38, etc.

[3] *Mem. Dept. Agric. India*, 1908, Vol. I. 79.

where $v$ and $l$ as before represent respectively the volume of the water and the length of the wetted soil (Fig. 2).
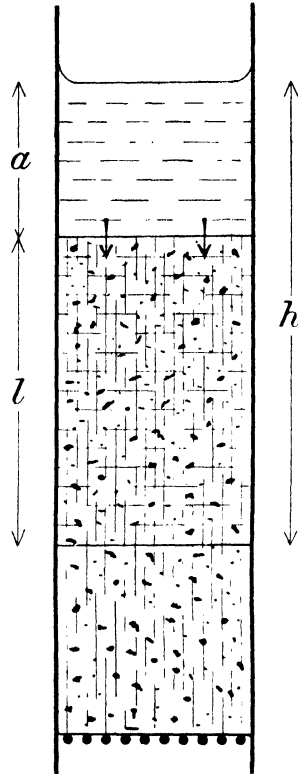


FIG. 2.

Then on the basis of equation (3), we get

$$\frac{dl}{dt} = \frac{\pi}{8\eta} \cdot \frac{gs\,(h + K)}{l} \cdot \frac{\Sigma r^4}{AS} \quad \dots\dots\dots\dots\dots(7)$$

$$= \frac{cp}{S} \cdot \frac{h + K}{l}$$

$$= \frac{P}{S} \cdot \frac{h + K}{l} \quad \dots\dots\dots\dots\dots\dots\dots\dots(8);$$

therefore as $h = a + l$, and $a$, $K$, $P$ and $S$ are constants,

$$\frac{P}{S}\,t = \int_l^0 \frac{l \cdot dl}{l + a + K};$$

and, since $t = 0$ when $l = 0$,

$$\frac{P}{S} t = l - (a + K) \log_e \left( 1 + \frac{l}{a + K} \right) \quad\cdots\cdots\cdots\cdots\cdots(9).$$

2. The second possibility comprises the case in which the water is rising vertically in the column of soil, under the influence of capillarity, and against the hydrostatic pressure.

If the lower end be just in contact with the water surface then $h = l$ and $a = 0$ and the equation becomes

$$\frac{dl}{dt} = \frac{P}{S} \cdot \frac{K - l}{l} \quad\cdots\cdots\cdots\cdots\cdots\cdots\cdots(10),$$

which on integration gives

$$\frac{P}{S} t = K \log_e \left( \frac{l}{K - l} \right) - l \quad\cdots\cdots\cdots\cdots\cdots(11).$$

3. In the third possibility the motion is horizontal, and in this case is due entirely to capillary suction.

Hence
$$\frac{dl}{dt} = \frac{P}{S} \cdot \frac{K}{l} \quad\cdots\cdots\cdots\cdots\cdots\cdots\cdots(12),$$

and integrating,

$$\frac{PK}{S} t = \tfrac{1}{2} l^2 \cdots\cdots\cdots\cdots\cdots\cdots\cdots(13).$$

This case is perhaps the most interesting, for not only is it often realized in the field (as when water is percolating from irrigation channels) but its experimental realization in the laboratory can be readily followed without the mathematical and other complications present in the two first cases.

§ 12. *Experimental tests of validity and further development of the formulae deduced in* § 11.—We have thus deduced three formulae representing the movement of water through dry soil in the three important directions, and the validity of these provisional formulae (9, 11, 13) has been tested experimentally in the following manner.

§ 13. A glass tube about one inch in diameter and thirty inches long was carefully and uniformly packed with air-dry loam to within six inches of the upper end; the lower end of the tube being closed with a disc of copper gauze supported by a perforated cork. A layer of water 10 cms. deep was placed on top of the soil column, and any deficiency caused by percolation of this water into the soil was made good by means of the usual constant level apparatus.

Readings were taken of the progress of the water downwards at intervals during sixty hours, at the end of which time water began to drip from the lower end of the tube.

From equation (9),

$$\frac{P}{S} = \frac{l - (a + K)\log_e\left(1 + \dfrac{l}{a + K}\right)}{t}.$$

$K$ was unknown, but a few trials showed that $K = 90$ gave the best results.

The observed values of $l$ were plotted against $t$, and the following results were obtained from the curve drawn through the experimental points:

TABLE I.

| $t$ (hours) | $l$ (centimetres) | $\dfrac{P}{S}$ |
|---|---|---|
| 0·25 | 3·3 | ·20 |
| 0·5 | 4·6 | ·20 |
| 1 | 6·7 | ·206 |
| 2 | 9·7 | ·210 |
| 3 | 11·9 | ·227 |
| 4 | 14·0 | ·225 |
| 5 | 15·6 | ·218 |
| 10 | 21·7 | ·208 |
| 20 | 31·2 | ·202 |
| 50 | 52·4 | ·206 |
| 60 | 58·0 | ·204 |

§ 14. The same concordance was not obtained, however, when equation (11) was applied by us to a series of measurements carried out by Loughridge[1] on the rate of rise of water in a tube of soil.

In this case $K$ was taken as 42·5, for that was the maximum height reached by the ascending water.

By equation (11),

$$\frac{K\log_e\left(\dfrac{K}{K - l}\right) - l}{t} = \frac{P}{S} = \text{a constant};$$

but the actual values calculated from the experimental readings shew that there is some factor which has not been taken into account.

TABLE II.

| $t$ (hours) | $l$ (centimetres) | $\dfrac{K \log \left( \dfrac{K}{K-l} \right) - l}{t}$ |
|:---:|:---:|:---:|
| 1 | 20·4 | 7·4 |
| 2 | 25·5 | 6·75 |
| 6 | 31 | 4·1 |
| 12 | 33 | 2·55 |
| 24 | 35·7 | 1·75 |
| 48 | 39 | 1·40 |
| ·144 | 42·5 | — |

§ 15. In these calculations the water has been regarded as at once occupying the whole of the pore-space in each layer of the soil as it reaches it. This condition is apparently realised in the case of a downward flow of water; but when the percolation is upward or horizontal numerous experimenters have shewn that the percentage of moisture in the soil decreases continuously with the distance from the water supply.

The same difficulty has been recognised by Bell and Cameron[1], who have also investigated the rate of entry of water into capillary spaces. They found that for a single horizontal capillary tube the velocity of movement is in accord with equation (13),

$$\frac{l^2}{t} = C,$$

but for soils and other porous media the rate of movement of the visible water front could only be empirically represented by the equation,

$$\frac{l^n}{t} = C',$$

where $n$ is generally greater than 2 but varies for each individual experiment.

They say: "In this latter case, however, there is no way of distinguishing between that portion of the substance which is merely wet by a capillary film of liquid over the grains or fibres, and that portion of the substance whose interstices are filled with the liquid."

§ 16. If, however, instead of modifying the foregoing equation by empirically altering $n$, it is modified in such a way as to take into account the fact that the length of moistened soil does not correspond

[1] *Jour. Phys. Chem.* 1906, x. 663.

with the real length of column traversed by the whole of the water, but is greater than this; then the difficulty will be found to disappear.

Consider the case of a horizontal soil column (in order to eliminate all but capillary forces), then the distribution of water will be as diagrammatically illustrated in Fig. 3, the foremost portion of the moist area being incompletely saturated and $\theta/s$, the fraction of the soil pores occupied by water, being less than unity.
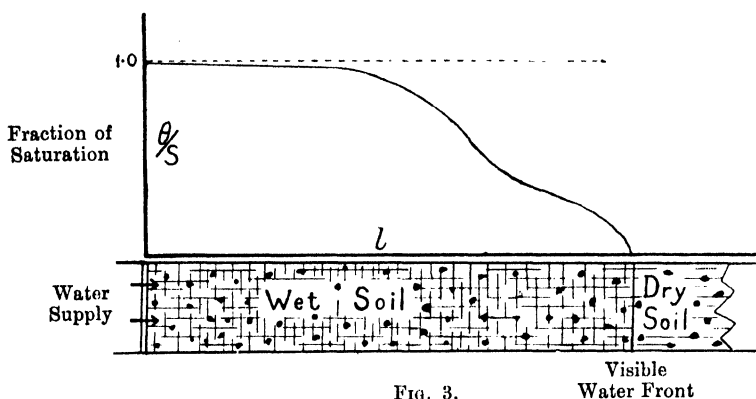


Fig. 3.

If we assume that the water present in this region is distributed uniformly among the fine and coarse capillaries then the average effective length of the soil column will be equal to $\dfrac{v}{AS}$, the volume of water which has entered the tube per unit area of cross-section of the soil pores. But $\dfrac{v}{AS} = \dfrac{m}{sAS}$ where $m =$ the mass of water absorbed by the soil column, and $s =$ density of water $= 1$ (with sufficient accuracy).

Substituting this quantity for $l$ in equation (13), $\dfrac{2KP}{S} = \dfrac{l^2}{t}$, we obtain

$$2A^2 KPS = \frac{m^2}{t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(14),$$

which should, even though not rigidly correct, give us a close approximation to the truth.

The following measurements were taken of both the rate of linear flow and the weight of water percolating into a soil tube:

TABLE III.

| t (hours) | $\dfrac{l^2}{t}$ | $\dfrac{m^2}{t}$ | $\dfrac{\theta}{S}$ |
|---|---|---|---|
| 1 | 61·3 | 25·3 | ·804 |
| 2 | 56·9 | 25·9 | ·848 |
| 3 | 55·5 | 26·0 | ·860 |
| 6 | 53·9 | 26·3 | ·877 |
| 9 | 52·3 | 26·3 | ·890 |
| 12 | 49·0 | 25·9 | ·915 |
| 18 | 48·5 | 25·7 | ·915 |
| 21 | 48·0 | 25·7 | ·915 |
| 24 | 48·1 | 25·9 | ·923 |
| 26 | 48·3 | 26·0 | ·923 |

The superiority of equation (14) over equation (13) is conclusively shewn; the values of $\dfrac{l^2}{t}$ and of $\theta/s$ (the fraction of the pore-spaces of the wet soil filled with water) are such as would be expected from the foregoing considerations. The slight rise shewn in the later values of $\dfrac{l^2}{t}$, and less markedly of $\dfrac{m^2}{t}$, has been observed in almost every experiment, and may be accounted for by the effect of the viscosity of air, which steadily diminishes as the tube becomes filled with water.

§ 17. The constancy of $\dfrac{m^2}{t}$ as given in Table III. justifies the conclusion that the determination of the weight of water entering a horizontal soil column will lead us to a reliable and sufficiently accurate method of measuring $KP_wS$, and since $S$ and $P_w$ for the same column of soil can be determined independently, $K$ can be evaluated by substituting their values in equation (14),

$$2A^2KP_wS = \frac{m^2}{}$$

*Experimental Investigation.*

§ 18. *The soils used.*—Three soils were used in these preliminary experiments.

*A.* A friable loam obtained from the grounds of the University of Melbourne, prepared by breaking up with a rubber pestle. It was spread out on paper to dry, when it was passed through a sieve of about 0·5 mm. mesh—the coarse grains and rootlets, etc., being rejected.

The moisture content of this air-dried loam was found to be 1·41 per cent. Its density was determined by weighing in a 50 c.c. specific gravity bottle with kerosene ($d\frac{18}{18} = 0.7993$).

9·796 grams of air-dry soil displaced 3·072 grams of kerosene and therefore had a specific gravity of 2·496. Similarly 14·851 grams of the same soil dried at 100° C. displaced 4·756 grams kerosene and had a specific gravity of 2·549. This observed difference is exactly that calculated from the water content of the air-dry soil.

B. A clay soil obtained from Werribee, Victoria, for use in a series of experiments in connexion with the influence of fertilizers on drainage.

This soil was only used in a few experiments.

C. A surface sand obtained from Canterbury, near Melbourne, which was sufficiently free from colloidal clay to serve as a type of a highly permeable soil.

§ 19. *Method of experimenting.*—For the purpose of experiment glass tubes were filled as uniformly as possible with the soil.

The difficulties involved in packing tubes with soil are well known and there is no satisfactory method of determining with what uniformity a tube of soil has been packed. Even two tubes having the same permeability may be very dissimilar in respect to the actual arrangement of the particles of soil.

The method of filling employed in these experiments is as follows: The end of the tube is first temporarily closed with a cork, then about two inches length of soil introduced at a time, and the tube rotated rapidly around its longitudinal axis and occasionally "dumped": too much jarring causes a visible separation of the smaller from the larger particles. In this manner the tube is filled; the other cork is then pressed home, and alternate rotation and jarring continued until there is no further sign of closer packing. These temporary corks are now removed and the soil is kept in position by accurately fitting discs of filter paper and fine copper gauze backed up by perforated cork plugs carrying glass connecting tubes. Finally the corks and tubes were sealed in position with ceresin or paraffin wax as in Fig. 4.

Direct measurements shewed that the resistance offered by the discs of filter paper and gauze was negligible compared with that of a few centimetres of soil.

§ 20. In the first experiment, the results of which have already been given in Table III., the tube used had a mean diameter of 3·23 cm. and was filled to a length of 58 cm.

As the amount of soil introduced into the tube was not weighed it was impossible to calculate the pore-space $S$ and so obtain an exact value for $P$, but it will be observed that the water had reached the lower end of the tube in 60 hours.

A similar tube was at the same time filled with the clay soil. Here the downward progress of the water was at first rapid, but after a few minutes became extremely slow, and the advancing line of moisture shewed less and less distinctly until it was eventually practically invisible. An indication was however obtainable that the water had reached the lower end of the column in about eleven months; this gives an approximate idea of the relative permeabilities of the loam and the clay.
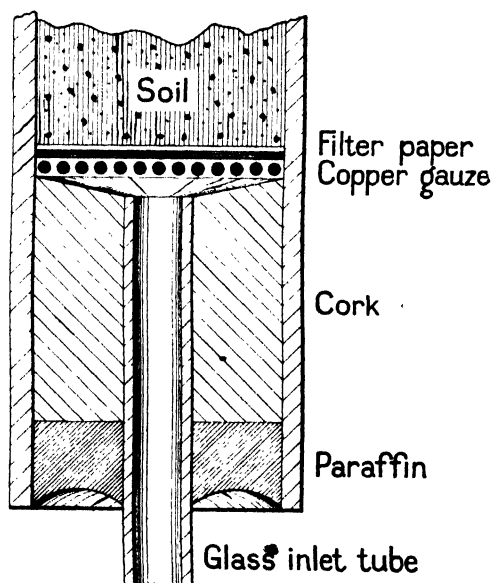


FIG. 4.

§ 21. *Procedure recommended for the "physical examination" of soils.*—In later experiments weighings were taken not only of the soil used but also of the water absorbed, and the following method of procedure was adopted for the "physical examination" of soils:

§ 22. (1) *Determination of the Specific Pore-Space* $(S)$.—The soil was filled into the tube in the manner described, and weighed; the length $(l)$ of the soil column was then measured and from the area of cross-section $(A)$ of the tube (previously determined from the water

content of a convenient length), and from the specific gravity of the soil, the value of $S$ can be calculated.

$$S = 1 - \frac{W}{Al\Delta},$$

where $W$ is the weight and $\Delta$ the specific gravity of the soil.

The value of $S$ affords interesting information as to the degree of compactness to which it is possible to pack the soil under the uniform conditions described.

§ 23. (2) *Determination of Permeability to Air.*—The soil tube is now attached by a short caoutchouc tube to an ordinary nitrometer containing water instead of mercury. An increased or diminished pressure can be obtained by appropriate adjustment of the water level in the two limbs and thus air may be forced through the soil tube in either direction.

Both pressure and suction are employed and the times of passage of certain volumes of air, as measured on the nitrometer scale, observed.

It is obvious that the pressure is constantly altering, but the relationship between the pressure (difference in levels) and the volume (indicated by the scale reading) is easily established for $h = h_0 \pm bv$, where $h_0$ is the initial pressure and $b$ is a constant for the particular nitrometer tubes employed.

From equation (5),

$$P_a = \frac{dv}{dt} \cdot \frac{l}{Ah},$$

and as $h = h_0 \pm bv$, then on integrating between the limits $h_1$ and $h_2$, *i.e.* $t_1$ and $t_2$; we get

$$P_a = \frac{\log_e \left(\frac{h_1}{h_2}\right) \cdot l}{(t_2 - t_1)\,Ab}$$

$$= \frac{2 \cdot 303 l}{A \cdot b} \cdot \frac{\log_{10} \left(\frac{h_1}{h_2}\right)}{t_2 - t_1} \quad \ldots\ldots\ldots\ldots\ldots(15).$$

The question as to whether concordant results could be obtained with either "head" or "tail" of pressure was conclusively answered by the following set of readings, and any corrections, such as are required when dealing with a single capillary tube (or higher pressures), are shewn to be unnecessary with ordinary soils for the low pressures used.

TABLE IV.

*Permeability of Soil to Air.*

| Using "head" of pressure | | | | Using "tail" of pressure | | | |
|---|---|---|---|---|---|---|---|
| $v$ (c.c. of air) | $t$ (minutes) | $h$ (cm. of water) | $\dfrac{\log_{10}\left(\dfrac{h_0}{h}\right)}{t}$ | $v$ (c.c. of air) | $t$ (minutes) | $h$ (cm. of water) | $\dfrac{\log_{10}\left(\dfrac{h_0}{h}\right)}{t}$ |
| 0 | 0 | + 32·2 | — | 0 | 0 | − 30·0 | — |
| 2 | ·51 | 29·0 | ·0892 | 2 | ·55 | 26·8 | ·0891 |
| 6 | 1·70 | 22·7 | ·0893 | 5 | 1·52 | 22·0 | ·0886 |
| 9 | 2·88 | 17·9 | ·0885 | 7 | 2·28 | 18·8 | ·0889 |
| 10 | 3·81 | 14·8 | ·0885 | 11 | 4·32 | 12·5 | ·0880 |
| 12 | 4·38 | 13·2 | ·0884 | 12 | 4·99 | 10·9 | ·0881 |
| 13 | 5·00 | 11·7 | ·0879 | 13 | 5·79 | 9·3 | ·0878 |
| 14 | 5·71 | 10·1 | ·0882 | 14 | 6·76 | 7·7 | ·0874 |
| 15 | 6·56 | 8·5 | ·0882 | 15 | 7·89 | 6·1 | ·0877 |
| 16 | 7·58 | 6·9 | ·0883 | | | | |
| | | | | | | Average... | ·0882 |
| | | Average... | ·0885 | | | | |

But by taking $h_1$ and $t_1$ as the initial pressure and time in each calculation any error at that point will affect every observation, and so in later experiments a fresh starting point was taken for each calculation.

§ 24.  A more convenient and accurate arrangement of apparatus was also devised in which the graduated limb of the nitrometer was replaced by an inverted burette with a two-way tap and the other limb by a large water reservoir.  The pressure corresponding to any burette reading is given by the difference in level of the water in the burette and in a short gauge tube of the same diameter attached to the connecting tube by a T-piece.  (See Fig. 5.)

In order to avoid inconveniently long or short times of flow the diameter or length of the tube used to contain the soil should be selected to suit its permeability.

The water level is first adjusted to the 25 c.c. mark on the burette with the tap open to the air, and then by applying suction the level is raised several centimetres above the 50 c.c. mark.  The tap is then turned to communicate with the soil tube, and the stop-watch started as the water passes the 45 c.c. mark and time readings taken over a range of 15 c.c. when the watch is stopped at the 30 c.c. mark.

Similarly a set of readings are taken between the 5 and 20 c.c.

marks, when the air will be drawn through the soil tube under a "tail" of pressure.
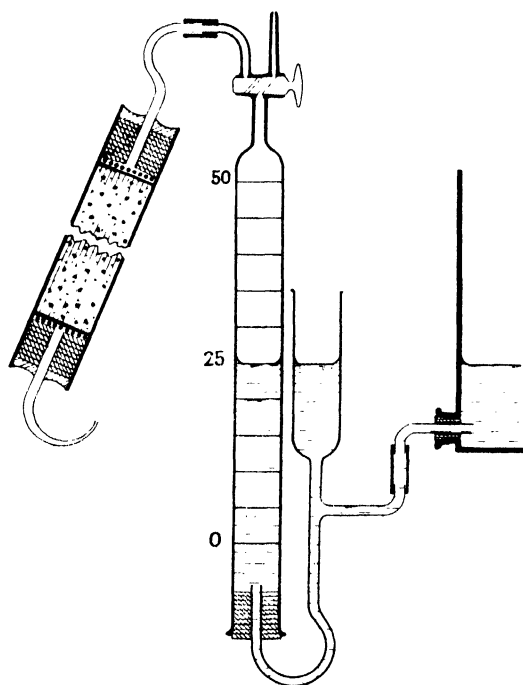


FIG. 5.

The observations recorded in Table V. were taken with both "head" and "tail" of pressure on a tube filled with loam.

Although values of $\dfrac{\delta v}{\delta t} \cdot \dfrac{1}{h}$ and consequently of $P_a$ can be obtained by taking the differences between consecutive readings in this way, a more systematic method of utilizing such a series of observations is that given in Table VI. As the values of $\log_{10}\left(\dfrac{h_1}{h_2}\right)$ have only to be determined once for each apparatus, this method of calculation is probably the simplest in practice and certainly the most satisfactory.

Considering the probable calibration errors of an ordinary nitrometer (such as was employed in these experiments) the *accuracy* of this method of determining the permeability of a column of soil is beyond criticism and the simplicity of the apparatus and measurements

required give it an important advantage over the methods usually employed for that purpose.

TABLE V.

*Calculation of Permeability Constant from Experimental Results.*

| $v$ (c.c.) | $t$ (minutes) | | | $h$ (cm.) | $\frac{\delta v}{\delta t} \cdot \frac{1}{h}$ |
|---|---|---|---|---|---|
| | Head | Tail | Mean | | |
| 0 | 0 | 0 | 0 | 31·68 | 0·1116 |
| 2 | ·600 | ·590 | ·595 | 28·52 | 0·1111 |
| 4 | 1·265 | 1·260 | 1·263 | 25·35 | 0·1110 |
| 5 | 1·63 | 1·63 | 1·63 | 23·77 | 0·1117 |
| 6 | 2·03 | 2·01 | 2·02 | 22·19 | 0·1127 |
| 7 | 2·44 | 2·43 | 2·435 | 20·36 | 0·1098 |
| 8 | 2·89 | 2·90 | 2·895 | 19·01 | 0·1109 |
| 9 | 3·38 | 3·40 | 3·39 | 17·42 | 0·1124 |
| 10 | 3·925 | 3·925 | 3·925 | 15·84 | 0·1099 |
| 11 | 4·51 | 4·55 | 4·53 | 14·26 | 0·1118 |
| 12 | 5·16 | 5·23 | 3·195 | 12·67 | 0·1107 |
| 13 | 5·89 | 6·02 | 5·955 | 11·09 | 0·1111 |
| 14 | 6·75 | 6·91 | 6·83 | 9·50 | 0·1109 |
| 15 | 7·71 | 8·02 | 7·865 | 7·92 | |
| | | | | Average... | 0·1112 |

$$P_a = \frac{l}{A \cdot 60} \cdot \frac{\delta v}{\delta t} \cdot \frac{1}{h} = \frac{35 \cdot 05 \times \cdot 1112}{1 \cdot 950 \times 60} = 0 \cdot 0333.$$

TABLE VI.

*Calculation of Permeability Constant from Experimental Results.*

| $v_1 \quad v_2$ | $t_1$ | $t_2$ | $t_2 - t_1$ | $\log_{10}\left(\frac{h_1}{h_2}\right)$ | $\dfrac{\log_{10}\left(\frac{h_1}{h_2}\right)}{t_2 - t_1}$ |
|---|---|---|---|---|---|
| 0— 9 | 0 | 3·390 | 3·390 | ·2597 | ·0764 |
| 2—10 | 0·595 | 3·925 | 3·330 | ·2554 | ·0766 |
| 4—11 | 1·263 | 4·53 | 3·287 | ·2499 | ·0763 |
| 5—12 | 1·63 | 5·195 | 3·565 | ·2732 | ·0764 |
| 6—13 | 2·02 | 5·955 | 3·935 | ·3012 | ·0765 |
| 7—14 | 2·435 | 6·83 | 4·395 | ·3353 | ·07623 |
| 8—15 | 2·895 | 7·865 | 4·970 | ·3803 | ·0766 |
| | | | | Average.. | ·07643 |

$$P_a = \frac{2 \cdot 303 l}{60 \cdot Ab} \cdot \frac{\log_{10}'\left(\frac{h_1}{h_2}\right)}{t_2 - t_1} \quad \frac{2 \cdot 303 \times 35 \cdot 05 \times \cdot 07643}{60 \times 1 \cdot 950 \times 1 \cdot 585} = \cdot 03325.$$

§ 25.  (3)  *Measurement of Capillary Flow.*—The tube is now placed in a horizontal position with the sickle-shaped inlet tube dipping into a dish of water, the surface of which is adjusted to the same level as the axis of the soil column.

A slight suction is now applied at the far end so as to fill the inlet tube with water.  Percolation immediately starts and its progress is measured at convenient intervals by weighing the soil tube rapidly and keeping it in the horizontal position so as not to interfere with the percolation.

The distance to which the visible water front has penetrated may also be measured, several readings being taken to obtain its mean position when its progress on different sides of the tube is irregular, as is the case when the packing is not uniform.

<div align="center">TABLE VII.</div>

*Calculation of Capillary Constants from Experimental Results.*

| Temp. | $t$ hours | Tube A | | | | Tube B | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $l$ | $m$ | $\dfrac{m^2}{t}$ | $\dfrac{\theta}{S}$ | $l$ | $m$ | $\dfrac{m^2}{t}$ | $\dfrac{\theta}{S}$ |
| 15°·1 | 0·5 | 5·56 | 3·44 | 23·7 | ·775 | 5·66 | 3·35 | 22·5 | ·717 |
| 15°·3 | 1 | 7·83 | 5·02 | 25·3 | ·805 | 7·82 | 4·73 | 22·4 | ·722 |
| 15°·4 | 2 | 10·67 | 7·20 | 25·9 | ·846 | 10·80 | 6·81 | 23·2 | ·754 |
| 14°·8 | 3 | 12·90 | 8·84 | 26·0 | ·859 | 13·12 | 8·44 | 23·8 | ·768 |
| 13°·8 | 4 | 14·69 | 10·17 | 25·8 | ·869 | 14·79 | 9·76 | 23·9 | ·788 |
| 13°·5 | 6 | 17·98 | 12·55 | 26·3 | ·875 | 17·50 | 11·99 | 24·0 | ·818 |
| 12°·6 | 9 | 21·66 | 15·38 | 26·3 | ·890 | 21·34 | 14·69 | 24·0 | ·822 |
| 11°·5 | 11·67 | 23·87 | 17·39 | 25·9 | ·913 | 24·22 | 16·59 | 25·6 | ·817 |
| 11°·9 | 14 | 25·98 | 18·97 | 25·7 | ·917 | 26·30 | 17·95 | 23·0 | ·815 |
| 11°·4 | 18 | 29·52 | 21·49 | 25·7 | ·914 | 29·16 | 20·35 | 23·0 | ·835 |
| 12°·8 | 21 | 31·75 | 23·23 | 25·7 | ·918 | 31·28 | 21·95 | 23·0 | ·838 |
| 13°·2 | 23·1 | 33·30 | 24·42 | 25·8 | ·920 | 32·58 | 23·05 | 23·0 | ·846 |
| 13°·3 | 25 | 34·73 | 25·47 | 25·9 | ·921 | 33·85 | 24·03 | 23·1 | ·848 |
| 13°·5 | 26·2 | 35·58 | 26·11 | 26·0 | ·920 | — | — | — | — |
| 13°·5 | 27 | — | — | — | — | 35·17 | 24·97 | 23·1 | ·848 |
| 13°·4 | 27·8 | — | — | — | — | 35·70 | 25·38 | 23·2 | ·850 |
| Saturated... | | — | 28·36 | — | 1·000 | — | 29·87 | — | 1·000 |
| | | — | | *25·85* | — | — | | *23·25* | — |

Tube A.    $KPS = \dfrac{m^2}{t} \cdot \dfrac{1}{2A^2} = \dfrac{25 \cdot 85}{3600 \times 2 \times (1 \cdot 950)^2} = 0 \cdot 000944.$

Tube B.    $KPS = \dfrac{m^2}{t} \cdot \dfrac{1}{2A^2} = \dfrac{23 \cdot 25}{3600 \times 2 \times (1 \cdot 962)^2} = 0 \cdot 000838.$

These measurements of $l$ are not required for the calculation of $K$, but are of interest as shewing the distribution of water in the "wet" soil.

§ 26.    The experiments detailed in Table VII. were carried out simultaneously on two tubes filled with loam in slightly different states of compactness, and illustrate the degree of accuracy to be expected.

The small discrepancies in the values of $\dfrac{m^2}{t}$ can be largely accounted for by the effect of changes of temperature[1] on the viscosity of the percolating water; another possibility of slight error is the uncertainty in the initial readings and the correction for capacity of the inlet tube.

The figures in the columns headed $\dfrac{\theta}{S}$ denote the fractional saturation of the wetted portion of the soil. Both tubes had been as uniformly and similarly packed as possible, but the difference in the observed values of $KPS$ is quite appreciable—0·00094 and 0·00084—and is due to the different values of $S$—0·475 and 0·4635.

§ 27.    (4)  *Saturation of the Soil in the Tube.*—After the whole soil column has become wetted the tube is turned up and the inlet (at the lower end) connected with a reservoir of water kept at a higher level than the top of the soil. After some time water begins to flow from the upper end of the soil, and, the outlet having been bent over and drawn out somewhat, this issuing water can be collected and weighed or measured without loss.

Weighings of the tube are made from time to time until no further increase is observed. This gives the amount of water required to saturate the soil and enables a comparison to be made with the value of $S$ as determined in § 22 from the volume and weight of the soil column. The discrepancy usually found is due to incomplete expulsion of the air from the interstices of the soil.

§ 28.    (5)  *Measurement of Permeability to Water.*—The water reservoir is now adjusted at a convenient height above the level of the outlet from the soil tube and the permeability of the soil to water ($P_w$) calculated from the rate of flow.

$$P_w = \frac{v}{t} \cdot \frac{l}{A h} \quad\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(5).$$

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[1] Recently some experiments have been carried out on tubes kept in a draught-cupboard whose temperature was maintained at 20° by means of a gas burner and thermostat. The values obtained for $\dfrac{m^2}{t}$ shewed the expected improvement.

The usual method of measuring permeability is to measure the downward flow, but the rate of flow under these conditions constantly decreases on account of the silting which takes place with ordinary soils. In one tube, the rate of flow became practically zero at the end of three months. This difficulty is overcome by causing the water to flow upwards through the soil and by only employing moderate pressures.

The figures given in Table VIII. were those actually obtained from two different tubes of similar soil.

## Table VIII.

*Comparison of Upward and Downward Flow of Water for Measurement of Permeability.*

| Downward Flow.    Pressure = 76·6 cm. | | Upward Flow.    Pressure = 20 cm. | |
| --- | --- | --- | --- |
| Time | Rate per hour | Time | Rate per hour |
| 1 hour | 7·85 c.c. | 7 hours | 0·32 c.c. |
| 4½ hours | 5·70 ,, | 21 ,, | 0·32 ,, |
| 21 ,, | 3·49 ,, | 67 ,, | 0·30 ,, |
| 68 ,, | 2·47 ,, | 96 ,, | 0·28 ,, |
| 90 ,, | ·80 ,, | | |
| 330 ,, | ·09 ,, | | |

§ 29. (6) *Statement of results.*—Having thus measured $S$, $P_w KS$ and $P_w$ directly, $K$ can be calculated and the three important constants ($S$, $P_w$ and $K$) controlling the flow of water through the soil should be tabulated.

The structure of its pore-spaces is more definitely described in this way than by any enumeration of the percentages of the various sized particles of which it consists.

§ 30. The permeability to air should also be tabulated, as on it depends the natural aeration of the soil, and in this connexion a further important relation is given by $\dfrac{\eta_a P_a}{\eta_w P_w}$. This ratio of the intrinsic permeabilities of soil to air and to water depends on the colloidal matter present and will vary from unity for a clean sand to fourteen or more for a clay soil packed as described above.

*Experimental Results for Three Typical Soils.*

§ 31. A number of experiments were carried out on these lines on each of the three soils described above and the results tabulated in Tables IX. and X.

### TABLE IX.

*The variation of Permeability with Specific Pore-space.*

| No. | Soil | Condition | $\theta$ | $S$ | $P_a$ |
|---|---|---|---|---|---|
| 1 | A.  University Loam | Moist | ·064 | ·493 | ·0356 |
| 2 | ,, | Air-dried | ·020 | ·445 | ·0334 |
| 3 | ,, | Dried at 100° | — | ·112 | ·0193 |
| 4 | ,, | Air-dried | ·0184 | ·490 | ·0433 |
| 5 | ,, | ,, | ·0192 | ·467 | ·0333 |
| 6 | ,, | ,, | ·0192 | ·466 | ·0393 |
| 7 | ,, | ,, | ·0193 | ·463 | ·0348 |
| 8 | ,, | ,, | ·0194 | ·461 | ·0296 |
| 9 | ,, | ,, | ·0196 | ·454 | ·0291 |
| 10 | ,, | Dried at 100° | — | ·495 | ·0450 |
| 11 | ,, | ,, | — | ·478 | ·0405 |
| 12 | ,, | ,, | — | ·475 | ·0315 |
| 13 | ,, | ,, | — | ·467 | ·0333 |
| 14 | ,, | ,, | — | ·4635 | ·0317 |
| 15 | B.  Werribee Clay | Air-dried | ·0302 | ·431 | ·0312 |
| 16 | ,, | ,, | ·0303 | ·428 | ·0336 |
| 17 | ,, | ,, | ·0305 | ·423 | ·0285 |
| 18 | C.  Canterbury Sand | Air-dried | ·0050 | ·379 | ·302 |
| 19 | ,, | ,, | ,, | ·378 | ·274 |
| 20 | ,, | ,, | ,, | ·373 | ·2795 |
| 21 | ,, | ,, | ,, | ·373 | ·251 |
| 22 | ,, | ,, | ,, | ·372 | ·245 |
| 23 | ,, | ,, | ,, | ·368 | ·233 |
| 24 | ,, | ,, | ,, | ·367 | ·262 |
| 25 | ,, | ,, | ,, | ·363 | ·252 |
| 26 | ,, | ,, | ,, | ·363 | ·248 |
| 27 | ,, | ,, | ,, | ·362 | ·248 |

Both the pore-space ($S$) and permeability ($P_a$) in Experiments 1—3 in Table IX. show clearly the effect of moisture on the packing of a soil.

The dependence of the permeability on the specific pore-space is clearly shown by Experiments 4—9, 10—14 and 18—27 in the same Table.

It will there be seen that the specific pore-space is not the only controlling factor, for the discrepancies are greater than can be accounted for by experimental error. This same phenomenon was also noticed in measurements on spherical glass beads, and it would appear that the system of arrangement of the particles has some influence on

## Table X.

*Summary of Experiments with Typical Soils.*

| | | University Loam | | | | | Werribee Clay | | | Canterbury Sand | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Specific pore-space | $S$ | ·495 | ·463 | ·467 | ·4635 | ·475 | ·431 | ·428 | ·423 | ·362 | ·363 |
| Length of soil column | $l$ | 35·4 | 36·0 | 35·05 | 35·7 | 35·6 | 37·6 | 38·5 | 37·15 | 73·8 | 67·45 |
| Area of cross-section | $A$ | 1·962 | 1·912 | 1·950 | 1·962 | 1·950 | 1·935 | 1·802 | 1·842 | 1·324 | 1·376 |
| Weight of soil | — | 88·46 | 91·99 | 91·69 | 94·93 | 92·72 | 107·8 | 103·2 | 102·7 | 162·8 | 152·8 |
| Permeability to air | $P_a \times 10^3$ | 45·0 | 34·8 | 33·3 | 31·7 | 31·5 | 31·2 | 33·8 | 28·6 | 248·0 | 252·0 |
| Percolation experiment | $\dfrac{m^2}{t} \times 10^3$ | 1·8 | 6·0 | 6·3 | 6·5 | 7·2 | 4·75 | 5·2 | 2·62 | — | 25·3 |
| " " | $KPS \times 10^3$ | — | — | — | ·84 | ·94 | — | — | — | — | — |
| Permeability to water | $P_w \times 10^3$ | ·182 | ·159 | ·175 | ·122 | ·081 | ·036 | ·045 | ·033 | 2·05 | 2·1 |
| Capillarity constant | $K$ | — | — | — | — | — | — | — | — | — | — |
| Ratio of intrinsic permeabilities as determined by air and water | $\dfrac{\eta_a P_a}{\eta_w P_w}$ | 3·5 | 3·6 | 3·1 | 4·1 | 6·0 | 14·2 | 12·3 | 14·2 | 2·0 | 2·0 |

the permeability as well as the average specific pore-space of the column.

§ 32. In considering these tables of results it must be borne in mind that none of the soils were in their natural condition; *e.g.* the loam samples had, with one exception, been dried and contained no moisture at the start of the experiment, whilst the clay had been air-dried and only contained 2·0 per cent. of water, and the sand had also been partially dried.

Notwithstanding this the different soils differ markedly in their constants, thus one characteristic feature shewn by the clay soils is the high value given for the ratio $\dfrac{\eta_a P_a}{\eta_w P_w}$, indicating that the water has the effect of swelling out the colloidal matter in the soil and so constricting the capillary passages. This ratio should be unity for soils composed of pure sand; experiments, on that medium and on glass beads, to be described in a subsequent paper, have given identical results for $\dfrac{\Sigma r^4}{A}$, whether calculated from $P_a$ or $P_w$ (*vide* § 9).

§ 33. Whilst more valuable when carried out on a natural soil *in situ*, these measurements of the percolation factors can also yield considerable information when applied to disturbed samples of soil, such as are ordinarily sent in to the laboratory for " analysis."

Only about one hundred grams are required, and it is suggested that the soil should be prepared by careful air-drying, and reducing to powder with a rubber pestle in the usual way when in a friable condition. The soil tube should then be packed as tightly and uniformly as practicable with this air-dry soil after all particles larger than 1 mm. in diameter have been removed.

## SUMMARY.

§ 34. 1. The permeability and capillarity constants of soil have been defined.

2. The movements of air and water through three types of soil have been measured and shewn to conform to equations connecting the rate of motion with the above constants.

3. It is suggested that the measurement of $S$, $P_a$, $P_w$ and $K$ is of more importance than, and should replace, the determination of the sizes of the soil particles as in the usual " mechanical analysis " of soils.

# THE NATURE, DISTRIBUTION AND EFFECTS UPON VEGETATION OF ATMOSPHERIC IMPURITIES IN AND NEAR AN INDUSTRIAL TOWN.

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## INTRODUCTION.

The following pages contain a summary of investigations and observations that have been made during the years 1906—1910, in part at the Manor Farm, Garforth (Experimental Farm of the University of Leeds and Yorkshire Council for Agricultural Education) and also in various parts of the city of Leeds.

The work comprises a three years' series of analyses of rain samples collected at Garforth, a twelve months' series of analyses of rain samples collected at the different stations in Leeds, and a variety of investigations and observations upon the effects of certain classes of atmospheric impurities upon vegetation. It will be convenient to deal with the results under these three heads.

## PART I. COMPOSITION OF RAIN COLLECTED AT GARFORTH.

The most extensive series of analyses of rain that have been made in this country are those carried out at Rothamsted[1]. According to the results there obtained the yearly rainfall carries down at Rothamsted on the average roughly $3\frac{3}{4}$ lb. per acre of nitrogen in the forms either of ammonia or nitric acid, $14\frac{3}{4}$ lb. of chlorine (as chlorides), and $17\frac{1}{2}$ lb. of sulphuric acid (total free and combined).

[1] For Summary of Results and Bibliography, see Miller, *Journ. Agr. Sci.* I. p. 280.

The Rothamsted results may probably be taken as typical of the rain falling in the essentially rural parts of the country, fairly remote from the sea, but will not hold good for the large tracts of agricultural land situated in the smoke-infested localities so extensive in area in many parts of the country.

With a view to supplementing the meagre information available as to the composition of rain falling in such districts, the collection of samples of rain was commenced at the Manor Farm, Garforth, in October, 1906, and continued without interruption until the close of the year 1909.

This farm is situated on the outskirts of the urban district of Garforth, some six miles due east of the great industrial city of Leeds, and on the north-eastern edge of the Yorkshire coal-field. The prevailing winds are westerly, and hence convey much of the impurity of the Leeds atmosphere over the farm. There is further local contamination from the coal-mining districts to the south and south-east, more particularly from two collieries situated respectively about one mile and half a mile from the centre of the farm. To the north-west, north, and north-east is a large tract of strictly rural character, so that from these quarters the atmosphere of the farm is not subject to serious contamination.

The samples were collected by means of two glass funnels, 12 inches in diameter at the rim, which were sunk so as to project only a few inches above the surrounding ground. The funnels were placed along-side the rain-gauge and other meteorological instruments, which are situated about 30 yards in front (to the east) of the dairy.

During the first twelve months (October, 1906—September, 1907) the rain collected was analysed as soon as it amounted to not less than 1000 c.c., but subsequently the analysis was deferred until about twice this quantity had accumulated. In every case where the rain collected during the period to which the sample referred was more than sufficient to fill the sample bottle, the whole of the rain collected during the period was mixed together and the bottle then filled. The sample was thus in each case representative of the whole rainfall of the period during which it was collected. It included, however, such suspended atmospheric impurities as deposited upon the funnels during the intervals between showers—a far from negligible contribution. The results obtained refer therefore to the whole of the impurities precipitated from the atmosphere upon the Garforth soil, whether by rain or otherwise.

The analysis included in every case estimations of nitrogen present as ammonia (free and combined) and as nitrate (+ nitrite); total chlorine present as chlorides; sulphur present as sulphates; and free acid.

From November, 1907, estimations were also made of sulphur present in states of oxidation lower than sulphate (sulphurous acid, etc.), and from January, 1908, of 'albuminoid ammonia.'

In all, 132 samples were dealt with. Details as to the range of variation in the composition of the samples are given in the Appendix, Table A. The results may be discussed conveniently on the basis of the averages for each month of the year. (Tables I.—V.)

The average rainfall (8″ gauge) for each month is given in Table I. The average yearly rainfall agrees closely with the average at Rothamsted and was fairly equally divided between the summer and winter half-years.

### Nitrogen.

The results with regard to the nitrogen-content of the rain are summarised in Tables I. and II.

Nitrogen present as ammonia (free and combined) was estimated by distillation with sodium carbonate and 'Nesslerisation' of the distillate; nitrogen present as nitrate by reduction with zinc-copper couple and estimation of the ammonia produced; 'albuminoid ammonia' (from January, 1908) by distillation with alkaline permanganate after removal of free and combined ammonia. The amount of nitrogen present in the form of organic matter would be certainly not less than that indicated by the result of the estimation of 'albuminoid ammonia.'

The main features of the results may be summarised as follows:—

(a) The total nitrogen in the forms of ammonia and nitric acid brought down by the rain (or contained in solid matters otherwise deposited) amounted, on the average of the three years, to 8·37 lb. per acre per annum, the average rainfall being 26·95 ins.

This amount is considerably higher than the Rothamsted average (13 years) of 3·84 lb., with an almost identical average rainfall (27·25 ins.)[1].

In addition to the ammoniacal and nitric nitrogen, a further quantity is brought down in the form of organic matter, this nitrogen

[1] Miller, this Journal, I. 281.

amounting at Garforth (2 years' mean) to fully 1·5 lb. per acre per annum.

The total precipitation of nitrogen in all forms would thus amount, on the average of the three years, to little short of 10 lb. per acre per annum.

## TABLE I.

*Nitrogen as Ammonia and Nitric Acid in Garforth Rain.*

Monthly averages.

(Oct. 1906—Dec. 1909.)

| | No. of Samples | Rainfall | Nitrogen | | | | |
| | | | Per Acre | | | Per cent. of Total | |
| | | | as $NH_3$ | as $N_2O_5$ | Total | as $NH_3$ | as $N_2O_5$ |
|---|---|---|---|---|---|---|---|
| | | inches | lb. | lb. | lb. | °/₀ | °/₀ |
| January | 3 | 1·03 | 0·32 | 0·10 | 0·42 | 76·6 | 23·4 |
| February | 6 | 1·08 | 0·31 | 0·07 | 0·38 | 82·7 | 17·3 |
| March | 10 | 2·52 | 0·69 | 0·16 | 0·85 | 80·6 | 19·4 |
| April | 10 | 2·26 | 0·75 | 0·34 | 1·09 | 68·8 | 31·2 |
| May | 15 | 2·66 | 0·68 | 0·17 | 0·85 | 79·5 | 20·5 |
| June | 11 | 2·80 | 0·42 | 0·12 | 0·54 | 77·6 | 22·4 |
| July | 10 | 2·76 | 0·53 | 0·21 | 0·74 | 71·4 | 28·6 |
| August | 8 | 2·65 | 0·57 | 0·09 | 0·66 | 86·9 | 13·1 |
| September | 6 | 1·34 | 0·49 | 0·28 | 0·77 | 64·0 | 36·0 |
| October | 24 | 3·33 | 0·74 | 0·21 | 0·95 | 77·5 | 22·5 |
| November | 15 | 1·92 | 0·47 | 0·07 | 0·54 | 86·7 | 13·3 |
| December | 14 | 2·60 | 0·46 | 0·11 | 0·57 | 80·9 | 19·1 |
| April—September | 60 | 14·47 | 3·44 | 1·21 | 4·66 | 74·0 | 26·0 |
| October—March | 72 | 12·48 | 2·99 | 0·72 | 3·71 | 80·5 | 19·5 |
| Whole year | 132 | 26·95 | 6·43 | 1·93 | 8·37 | 76·9 | 23·1 |

(*b*) The amount of nitrogen brought down in the course of the year is largely determined by the amount of rain. This is clearly evident in the data given below :—

| Year | Rainfall | Nitrogen (Ammoniacal + Nitric) |
|---|---|---|
| | inches | lb. per acre |
| 1907 | 27·1 | 8·45 |
| 1908 | 22·7 | 7·46 |
| 1909 | 28·5 | 9·48 |

This interdependence is not so clearly marked in the monthly averages, owing to the disturbing influence of temperature. Thus in 1907 the summer (April—September) rainfall, although somewhat less

TABLE II.

*'Albuminoid Nitrogen' in Garforth Rain.*

Monthly averages.

(Jan. 1908—Dec. 1909.)

|  | No. of Samples | Rainfall | Nitrogen per acre |
|---|---|---|---|
|  |  | inches | lb. |
| January | 2 | 1·10 | 0·09 |
| February | 3 | 0·93 | 0·07 |
| March | 5 | 3·06 | 0·30 |
| April | 6 | 2·81 | 0·41 |
| May | 6 | 2·45 | 0·07 |
| June | 3 | 2·38 | 0·05 |
| July | 4 | 2·99 | 0·09 |
| August | 4 | 2·91 | 0·06 |
| September | 4 | 1·80 | 0·24 |
| October | 5 | 1·70 | 0·05 |
| November | 2 | 0·64 | 0·01 |
| December | 5 | 2·82 | 0·12 |
| April—September | 27 | 15·34 | 0·92 |
| October—March | 22 | 10·25 | 0·64 |
| Whole year | 49 | 25·59 | 1·56 |

in amount than the winter (October—March) rainfall, contained appreciably more nitrogen, the difference being entirely in the ammoniacal nitrogen.

Taking the averages of the three years the highest monthly rainfall (October) coincided with the second highest precipitation of nitrogen, whilst the two lowest monthly rainfalls (January, February) coincided with the two lowest amounts of nitrogen.

Light rains were proportionately richer in nitrogen—especially in the form of ammonia—than heavy rains.

In all these respects the Garforth results are closely in agreement with those obtained at Rothamsted.

(c) The average ratio of ammoniacal to nitric nitrogen (Table I.) for the whole year was much the same at Garforth (76·9 : 23·1) as at Rothamsted (70·6 : 29·4).

At Rothamsted the ratio was appreciably higher for the summer than for the winter rain, but this held good at Garforth for only one year (1907) of the three.

### *Sulphur.*

The results with regard to the sulphur-content of the rain are summarised in Table III.

<div align="center">

TABLE III.

*Sulphur in Garforth Rain.*

Monthly averages.

</div>

| | As Sulphate or Free Sulphuric Acid (Oct. 1906—Dec. 1909) | | In other forms oxidisable to Sulphate (Nov. 1907—Dec. 1909) | | Total | Percentage distribution of Total Sulphur | |
|---|---|---|---|---|---|---|---|
| | $SO_3$ | | $SO_3$ | | $SO_3$ | as Sulphate | in other forms |
| | Parts per million | lb. per acre | Parts per million | lb. per acre | lb. per acre | | |
| January...... | 13·98 | 3·26 | 3·27 | 0·77 | 4·03 | 80·9 | 19·1 |
| February ... | 12·81 | 3·13 | 0·98 | 0·24 | 3·37 | 92·9 | 7·1 |
| March ...... | 9·82 | 5·60 | 2·87 | 1·63 | 7·23 | 77·4 | 22·6 |
| April ........ | 9·32 | 4·77 | 6·06 | 3·10 | 7·87 | 60·6 | 39·4 |
| May ........ | 9·90 | 5·96 | 4·02 | 2·43 | 8·39 | 71·0 | 29·0 |
| June ........ | 7·35 | 4·66 | 4·14 | 2·62 | 7·28 | 64·0 | 36·0 |
| July ........ | 11·48 | 7·17 | 5·20 | 3·25 | 10·42 | 68·8 | 31·2 |
| August ...... | 16·55 | 9·93 | 3·41 | 2·05 | 11·98 | 82·9 | 17·1 |
| September... | 13·72 | 4·16 | 9·69 | 2·94 | 7·10 | 58·6 | 41·4 |
| October ...... | 13·26 | 10·23 | 3·29 | 2·54 | 12·77 | 80·1 | 19·9 |
| November ... | 11·60 | 5·04 | 3·89 | 1·07 | 6·11 | 82·5 | 17·5 |
| December ... | 10·87 | 6·40 | 4·50 | 2·73 | 9·13 | 70·1 | 29·9 |
| April— Sept. | 11·39 | 36·65 | 5·42 | 16·39 | 53·04 | 67·6 | 32·4 |
| Oct.—March | 12·06 | 33·66 | 3·13 | 8·98 | 42·64 | 80·6 | 19·4 |
| Whole year | 11·72 | 70·31 | 4·27 | 25·37 | 95·68 | 74·2 | 25·8 |

Up to November, 1907, only sulphur present as sulphate was estimated, but from that date onwards the analyses were extended to include estimations of the sulphur present in other forms oxidisable to sulphate by digestion with bromine-water.

The average annual precipitation of sulphur was equivalent to 95·7 lb. $SO_3$ per acre, or more than five times the average at Rothamsted (17·41 lb.). The amounts were consistently high in each year, the

sulphur present as sulphate ranging from 64·28 lb. SO$_3$ per acre in 1908 to 72·54 lb. in 1909 (65·73 lb. in 1907), whilst the sulphur present in other forms was for these two years equivalent to 22·82 lb. and 27·91 lb. SO$_3$ per acre respectively. Thus, in each case about 26 per cent. of the total sulphur was present in forms less highly oxidised than sulphates,—a proportion which is indicative of high local contamination.

The proportion was decidedly higher for the summer half-year than for the winter, but there was little difference in the total sulphur, the summer total being only slightly higher than that for the winter—an observation in accord with results obtained at Rothamsted and elsewhere.

*Chlorine.*

The results with regard to chlorine are summarised in Table IV.

TABLE IV.

*Chlorine in Garforth Rain.*

Monthly averages.

(Oct. 1906—Dec. 1909.)

|  | lb. per acre |
|---|---|
| January | 3·26 |
| February | 1·60 |
| March | 1·51 |
| April | 1·67 |
| May | 1·22 |
| June | 1·46 |
| July | 1·50 |
| August | 1·99 |
| September | 0·86 |
| October | 1·69 |
| November | 1·43 |
| December | 2·70 |
| April—September | 8·70 |
| October—March | 12·19 |
| Whole year | 20·89 |

The average yearly precipitation of chlorine was 20·89 lb. as compared with the Rothamsted average of 14·87 lb., and maximum of 21·19 lb.

The totals for the three years were remarkably similar (20·27 lb., 20·16 lb., 19·39 lb.).

The winter rain, as at Rothamsted, was decidedly richer in chlorine than that falling during the summer months. Light rains brought down proportionately more chlorine than heavy rains. Thus the monthly rainfalls of less than 1 inch contained on the average 6·88 parts per million of chlorine, as compared with the average of 2·24 parts per million in the case of monthly rainfalls of 4 inches or more.

### *Free Acid.*

The impure nature of the Garforth atmosphere is strikingly illustrated by the amounts of free acid brought down in the rain.

The results obtained by titration with $\dfrac{N}{100}$ alkali, using methyl orange as indicator, are summarised in Table V.

TABLE V.

*Free Acid in Garforth Rain.*

Monthly averages.

(Oct. 1906—Dec. 1910.)

|  | Free Acid expressed as $H_2SO_4$ (lb. per acre) |
|---|---|
| January ............... | 1·50 |
| February............... | 1·10 |
| March ................. | 3·16 |
| April ............... .. | 1·07 |
| May ..................... | 1·59 |
| June ................. | 0·71 |
| July ..................... | 1·95 |
| August ............... | 0·56 |
| September ............ | 0·93 |
| October ............... | 1·69 |
| November ............ | 1·78 |
| December ........... | 4·01 |
| April—September ... | 6·81 |
| October—March ... | 13·24 |
| Whole year............ | 20·05 |

It will be noted that, on the average of the three years, the rain carried down free acid equivalent to no less than 20 lb. of sulphuric

acid per acre yearly, the amounts for the individual years being
15·62 lb., 28·32 lb., and 17·03 lb. respectively.

The winter rainfall was more than twice as acid as the summer
rainfall, the proportion being notably high in the months of December
and January. This is probably in large measure due to the prevalence
of fogs during these months, since the acidity was always high during
fogs. During the notoriously foggy month of December, 1908, the
acidity of the rain rose to the extraordinarily high figure of 41·4 parts
per million. The high acidity during such periods was accompanied
further by relatively large amounts of the other impurities.

In reviewing the Garforth results as a whole the outstanding
feature is the relatively high amount of each of the different impurities
estimated. Apart from the differences in this respect the records are
in substantial agreement with the Rothamsted results. There can be
no doubt that much of the impurity in the Garforth atmosphere is of
strictly local origin. The results bear unmistakable evidence of the
fact that the impurities have their origin largely in the combustion of
coal, this being the most obvious source of the acid which occurs
in such appreciable quantities in the rain. Very large areas of such
smoke-infested agricultural land are to be found in the north, and it
is not unlikely that the results obtained at Garforth will prove fairly
generally applicable to such areas.

PART II.    COMPOSITION OF RAIN COLLECTED IN VARIOUS PARTS
OF THE CITY OF LEEDS.

The extensive pollution of the Garforth atmosphere, as revealed
from the very outset of the investigations, suggested a diagnosis upon
similar lines of the atmosphere in various parts of the neighbouring
city of Leeds, which might fairly be regarded as responsible for some, if
not indeed for the greater part, of the impurities.

Accordingly ten representative stations were selected in localities of
different types, ranging from the markedly industrial to the purely resi-
dential suburban quarter. The accompanying plan (Fig. 1) indicates
the relative situation of the different stations and the character of the
area in which each was located.

During the twelve months[1] from November, 1907, to October, 1908,
samples of all rain falling at each station were collected and analysed,

[1] See footnote to Table VI.

## TABLE VI.

### *Impurities in Leeds Rain.*

### (Pounds per acre per annum*.)

| No. | Collecting Station | Period of Observation | Suspended matters | | | |
|---|---|---|---|---|---|---|
| | | | Total | Ash | 'Tar' (Ether-soluble matter) | 'Soot' (Ether-insoluble combustible matter) |
| | | months | lb. | lb. | lb. | lb. |
| 1 | Leeds Forge......... | 7 | 1886 | 1113 | 110 | 663 |
| 2 | Hunslet.............. | 6 | 1565 | 655 | 69 | 841 |
| 3 | Beeston Hill......... | 6 | 1163 | 709 | 149 | 305 |
| 4 | Philosophical Hall. | 12 | 849 | 423 | 78 | 348 |
| 5 | Headingley ... ... | 3 | 659 | 199 | 43 | 417 |
| 6 | Armley .............. | 7 | 598 | 216 | 34 | 343 |
| 7 | Woodhouse Moor... | 12 | 399 | 146 | 32 | 221 |
| 8 | Kirkstall ............ | 7 | 352 | 141 | 28 | 183 |
| 9 | Weetwood Lane ... | 12 | 147 | 54 | 26 | 67 |
| 10 | Roundhay........... | 6 | 90 | 49 | 14 | 27 |
| 11 | Garforth ............ (Nov.'07—Oct.'08) | 12 | — | — | — | — |

| Station No. | Free Acid expressed as $H_2SO_4$ | Sulphur | | | Chlorine | Nitrogen | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | in form of Sulphate $SO_3$ | in other forms $SO_3$ | Total | | as Ammonia N | as Nitrate N | as 'Albuminoid' N | Total |
| | lb. | lb. | lb. | lb. | lb. | lb. | lb. | lb. | lb. |
| 1 | 35 | 123 | 43 | 169 | 164 | 13·0 | 0·0 | 4·7 | 17·7 |
| 2 | 90 | 185 | 30 | 215 | 198 | 15·5 | 0·0 | 2·9 | 18·4 |
| 3 | 30 | 269 | 67 | 336 | 101 | 14·4 | 0·5 | 3·5 | 18·4 |
| 4 | 45 | 149 | 48 | 197 | 75 | 14·4 | 0·3 | 2·2 | 16·9 |
| 5 | 11 | 118 | 40 | 158 | 41 | 11·1 | 1·1 | 0·8 | 13·0 |
| 6 | 29 | 110 | 46 | 156 | 108 | 9·9 | 1·0 | 3·2 | 14·1 |
| 7 | 26 | 85 | 49 | 134 | 51 | 8·4 | 0·8 | 1·6 | 10·8 |
| 8 | 8 | 77 | 70 | 147 | 57 | 7·7 | 0·2 | 2·3 | 10·2 |
| 9 | 11 | 82 | 16 | 98 | 34 | 8·3 | 1·1 | 2·1 | 11·5 |
| 10 | 0 | 53 | 20 | 73 | 38 | 5·8 | 0·7 | 1·3 | 7·8 |
| 11 | 28 | 65 | 26 | 91 | 22 | 5·0 | 3·2 | 1·1 | 9·3 |

* For those stations where the sampling covered only a portion of the year the probable yearly total has been estimated by comparison with the average of stations 4, 7, and 9, at which the sampling covered the whole twelve months.

the procedure being that followed in connexion with the Garforth samples (p. 26). Further estimations were made of the total suspended matters, and of the 'tar' (ether-soluble matter) and ash contained therein. The matters other than 'tar' and ash are tabulated as 'soot.'
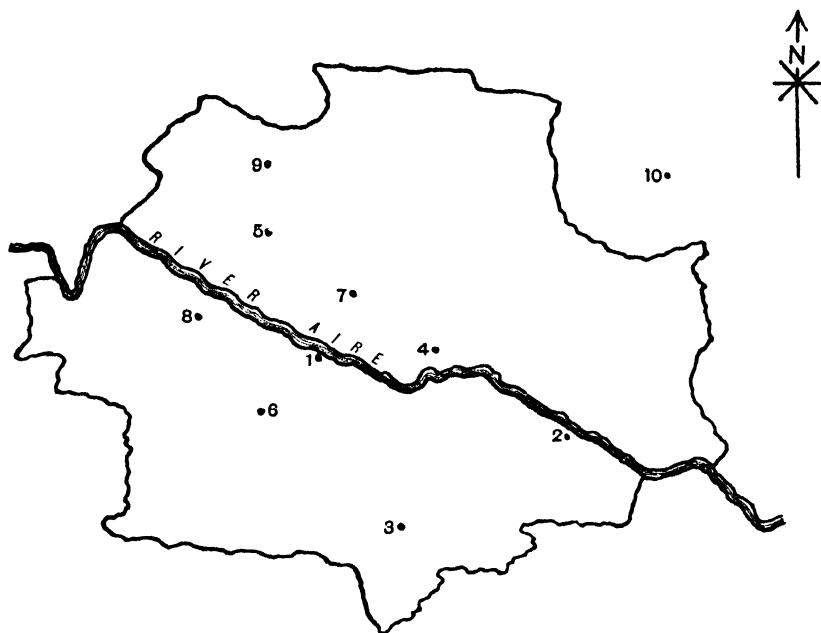


FIG. 1.

Plan of Leeds showing Stations.

Industrial—Stations 1, 2, 4.　　　Town Residential—Stations 6, 7.
Suburban Residential—Stations 3, 5, 8, 9, 10.

The totals (lb. per acre) for the twelve months at each station are given in Table VI., and are represented graphically in Fig. 2. The suspended impurities are plotted on a scale one-seventh of that used for the amounts of dissolved impurities other than nitrogen.

Details as to the range of variation in the composition of the samples collected at each station will be found in the Appendix, Table B, p. 55.

### Total Suspended Matter.

The influence of the industrial centres upon the amount of suspended impurities stands out most conspicuously. Thus at station 1
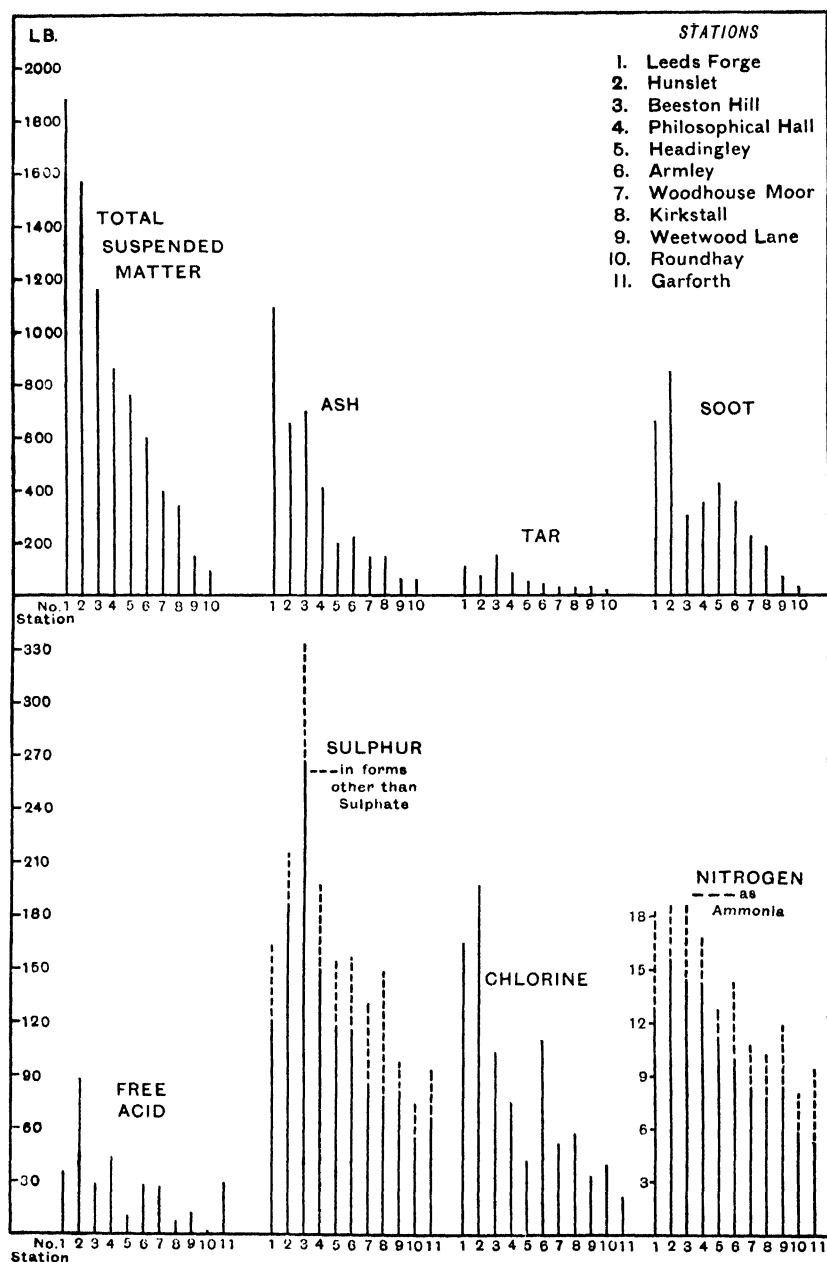
FIG. 2.

Impurities in Leeds Rain.

they amounted to nearly 1900 lb. per acre, at station 2 to roughly 1550 lb. per acre, whereas at the suburban station 10 they amounted to but 90 lb. In other words the suspended impurities were roughly twenty times as abundant in the chief industrial centres as in the purer atmosphere of station 10, some three miles due north-east of the centre of the city.

Leeds lies on the north-eastern fringe of the Yorkshire coal-field, the industrial area being almost entirely confined to the southern half of the city, whilst other industrial towns and coal-mining villages lie not far distant on this side in all directions ranging from south-east round by south to west. To the north and east the city merges into a vast tract of purely agricultural country. These facts explain the rapid diminution in the amount of suspended impurities as we pass northwards from the centre of the city. This is strikingly shown by the records of stations 4, 7, and 9. At station 4, in the heart of the city, the suspended impurities amounted to about 850 lb. per acre, whilst at station 7, less than a mile away to the north, they fell to 400 lb., or less than one-half, and at station 9, about two and a half miles to the north, they amounted to only 150 lb. per acre, or little more than one-sixth of the deposit in the centre of the city.

The prevailing winds in Leeds are west and south-west. It is to be expected therefore that the general drift of the impurities will be more towards the east than towards the west. This is well illustrated in the case of stations 5 and 8. Both are roughly the same distance from the centre of the city, both lie to the north-west, both overlook the industrial Kirkstall section of the Aire valley, station 5 from the east and station 8 from the west. It is interesting therefore to find that whilst the suspended impurities at station 5 amounted to 660 lb. per acre, they were only 350 lb. per acre across the valley at station 8.

## Ash.

The amount of ash varied greatly. It was remarkably high at station 1, which was situated in the grounds adjoining the Leeds Forge Company's works. The 'ash' contained in the solid matters precipitated there represented no less than 1100 lb. per acre per annum, or 60 per cent. of the total solids.

At the almost rural stations 9 and 10 the ash amounted to about 50 lb. per acre (= 45 per cent. of total), but at all the other stations nearer to the industrial area, the amount was notably higher.

## Tar.

The objectionable character of the matter suspended in the atmosphere of a city like Leeds is greatly intensified by its peculiar adhesiveness which arises from the presence in it of material of a tarry nature. An estimate of the amount of this 'tar' was made by extracting the suspended matters with ether. At all stations an appreciable amount of 'tar' was found in the suspended matter, the amount being greatest in and near the industrial area, and diminishing rapidly towards the north. Thus at station 4 in the centre of the city the yearly deposit of tar was computed at 80 lb. per acre ; one mile to the north, at station 7, it was 32 lb. or less than one-half; whilst at station 9, $2\frac{1}{2}$ miles to the north, it fell to 25 lb., and at station 10, 3 miles to the north-east, to 14 lb. only. It is interesting to note, however, that the suspended matters in the suburban areas, though smaller in amount, are much richer in 'tar' than those of the industrial areas. Thus, whereas at the suburban stations 9 and 10 the 'tar' formed 17 per cent. and 15 per cent. respectively of the total suspended matters, the proportion in the case of the industrial stations 1 and 2 was only 6 per cent. and 4 per cent. respectively. There is thus a characteristic difference between the contamination arising from the factory shaft and that attributable to the very imperfect combustion of the domestic range.

## Soot.

The term 'soot' is applied here to the combustible suspended matters that are insoluble in ether. The amount of such 'soot' was found to vary enormously at the different stations, ranging from a maximum of 840 lb. per acre yearly (equivalent to about 300 tons per square mile) at station 1 to 27 lb. at station 10.

The average of the five stations (Nos. 1, 2, 3, 4 and 7) situated within the central four square-mile area, amounted to about 190 tons of 'soot' per square mile per annum, or, roughly, $\frac{1}{2}$ ton per square mile per day of 'soot' actually reaching the ground within this area. According to the earlier investigation of Cohen[1] the total amount of soot thrown into the atmosphere is probably 40 times this amount.

The presence of these large quantities of suspended matters in the atmosphere has a very marked effect upon the amount of bright sunshine enjoyed in the centre of the city. Thus, to take the year 1907 as

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[1] Abs. *Journ. of the Sanitary Institute,* xviii. 607.

an example, the number of hours of bright sunshine registered at station 4 in the centre of Leeds was 1167 as compared with 1402 at Adel, some four miles to the north; in other words, in the centre of the city the duration of bright sunshine was curtailed by fully 17 per cent. The records for other years tell a similar tale. Particulars of experiments made with the object of obtaining more precise information upon this point will be found in Part III.

### Free Acid.

The majority of the samples of rain collected at the different stations were decidedly acid to methyl orange, indicating the presence of free mineral acid, probably mainly sulphuric acid arising from the combustion of coal. The distribution and amount of this free acid are somewhat erratic owing to the presence in the atmosphere also of impurities of alkaline reaction. Thus, the marked discrepancy between the acidities of the rain at the two main industrial stations (Nos. 1 and 2) is attributable to the large amount of alkaline impurities emitted by the furnaces near which the former station was located. At this station indeed the rain was in some cases appreciably alkaline in reaction. It is not difficult, however, to trace some degree of correlation between industrial activity and atmospheric acidity.

Free acid must be actively detrimental to vegetation, both by its direct action upon the leaves and by its slower action upon the soil. Experiments made to obtain information upon the latter point are dealt with in Part III.

The deposition of acid along with soot upon the leaves of plants is probably one of the chief causes of the early withering which is so characteristic of many forms of town vegetation, notably trees.

Ash trees in the purer parts of Leeds often retain their leaves some four or even six weeks longer than those in the more contaminated districts. Thus during the autumn of 1908, near station 10, with total suspended matter 90 lb. per acre and acidity nil, ash trees were found in full leaf on November 17th; near station 9, with suspended matter 147 lb., and acidity equal to 11 lb. $H_2SO_4$ per acre, the leaves had all fallen before the end of October, though a few were found still in leaf on the 22nd of that month; at station 8, with suspended matter 352 lb. and acidity equal to 8 lb. $H_2SO_4$ per acre, one ash tree only could be found in leaf on October 5th, whilst at station 5, with suspended matter 659 lb. and acidity 11 lb. per acre, all leaves had fallen from the ash trees on that date.

The acidity, as at Garforth, was remarkably high during fogs.   One sample of hoar-frost collected near station 5 during the dense fog of January 27th, 1909, contained free acid equivalent to no less than 103 parts of sulphuric acid per million, or more than fifty times the average acidity of the rain collected at this station.

## Sulphur.

The total sulphur was everywhere high, but notably so in and near the chief manufacturing areas.   The differences were, however, relatively much smaller than in the case of the other impurities.   The bulk of the sulphur was present in the form of sulphuric acid or sulphates, but in every case an appreciable amount was also present in other forms (*e.g.* $SO_2$, $SH_2$) readily oxidisable to sulphates.   The proportion of sulphur in these latter forms was much the same at all the ' city ' stations, but was much lower at the suburban stations 9 and 10.

## Chlorine.

The amounts of chlorine (present as chlorides) found in the yearly rainfall ranged from 34 lb. per acre at station 9 to practically 200 lb. (equivalent to about 3 cwt. of salt) at station 2.   Throughout the semi-industrial and non-industrial area the amount was not more than about 50 lb. per acre, with the exception of station 6, where the total reached 108 lb.   This high figure finds an explanation in the salt-glazing process carried on at a neighbouring fire-clay works.

The local origin of the bulk of the chlorides is evident from the fact that they diminish rapidly in quantity as we pass northwards from the centre of the city (compare stations 4, 7 and 9).   Moreover, the average chlorine-content of the samples collected at the industrial station 2 was about 35 parts per million, or nearly ten times the proportion found in the rain falling in rural districts.   Of the 200 lb. of chlorine deposited per acre yearly round this station, probably all but about 20 lb. must be attributed to local sources.

## Nitrogen.

The amounts of nitrogen ranged from about 8 lb. per acre at station 10 to $18\frac{1}{2}$ lb. each at stations 2 and 3.   The differences are thus not so striking as in the case of chlorine, free acid, and suspended matters, but are nevertheless considerable.   The lower figure agrees closely with the amount of nitrogen ($9\frac{1}{2}$ lb.) found to be precipitated at Garforth during the year in question.

In all cases the bulk of the nitrogen was present in the form of ammonia or ammonium compounds.   Indeed it was only well away from the centre of the city that the presence of nitrates in the rain could be detected at all.   The organic nitrogen, on the contrary, was most abundant in the industrial area.

The results summarised in the preceding pages leave no doubt as to the highly impure character of the atmosphere over the greater part of the city.   The influence of these conditions upon the health and material welfare of the citizens must be incalculable, but this aspect of the question is outside the scope of our investigations, which have dealt solely with the influence of the atmospheric impurities upon vegetation.   Upon this point a number of experiments have been carried out which remain to be described.

PART III.   INFLUENCE OF ATMOSPHERIC POLLUTION UPON PLANT GROWTH.

Atmospheric impurities may exercise a detrimental influence upon plant growth in a variety of ways.   The suspended insoluble impurities may impede growth

(a) By reducing the intensity of the sunlight incident upon the leaves.

(b) By blocking up some of the stomatal openings and thereby impeding the gaseous exchanges upon which assimilation and respiration depend.

(c) Possibly by the toxic influence of certain constituents of the suspended matters, e.g. sulphides.   This influence may be exercised directly upon the plant or indirectly through the soil.

The influence of the soluble impurities will be determined by their nature and amount.   There is no reason to believe that the nitrogenous impurities will be other than beneficial in their effects, and the same applies perhaps to the chlorides and sulphates, although here the quantities brought down by the rain may be excessive.   The only obviously detrimental soluble impurities are the free acid and the lower sulphur compounds ($SO_2$, $H_2S$, etc.), the latter being highly toxic in character.

*Influence of Smoke-laden Atmosphere upon Intensity of Light.*

Reference has already been made (Part II., p. 39) to the great difference between the amounts of bright sunshine recorded in the

centre of Leeds and outside its northern boundary. The 'sunshine-recorder,' however, takes no account of variations in the intensity of sunlight below a certain arbitrarily-fixed standard. If these were included in the comparison still greater differences might be expected. A convenient means of obtaining such more complete measurements is afforded by the action of light on solutions of potassium iodide, whereby iodine is liberated in quantities that are roughly proportional to the intensity of the light. This method was used by Cohen in 1896 for daily measurements at three stations in Leeds. A mixture of 50 c.c. of a 1 per cent. solution of potassium iodide and 10 c.c. of dilute sulphuric acid was exposed and at the end of the day the liberated iodine was estimated by titration with standard thiosulphate solution. Cohen found that in the heart of the industrial area (near station 1) the smoke pall absorbed about 25 per cent. of the total daylight.

Similar 'daylight tests' were made in the course of the present investigations simultaneously at six stations, the tests extending over several weeks. The results show a striking correlation between the amounts of suspended matters in the air and the intensity of the light. In Fig. 3 the six stations are arranged in *descending* order with reference to the amount of suspended impurities, and it will be noted that this is precisely the *ascending* order with reference to the intensity of the light. It will be observed that at station 2 with a yearly deposition of solid matter estimated at 1500 lb. per acre the intensity of the light was only sufficient to liberate daily an amount of iodine equivalent to 3·2 c.c. of $\frac{N}{10}$ thiosulphate, whilst at station 9, with suspended matter equivalent to only 150 lb. per acre the iodine liberated daily during the same period from a similar solution corresponded to 5·4 c.c. of the thiosulphate solution. In other words, at station 2 in one of the chief industrial areas, fully 40 per cent. of the light was shut out. Strictly speaking, of course, the iodine method only measures the variation in the intensity of the chemically active radiation, and the results do not necessarily hold good for the less refrangible rays which are of even greater importance to plants. It is highly probable, however, that the absorption by smoke will be much the same for rays of all wave-lengths. Evidence confirmatory of this view was obtained by measurements of the coefficients of absorption for red and violet light respectively of two suspensions of soot. The absorption was a little higher for the red than for the violet light, the ratio (red : violet) being practically the same in each case (120 : 100 and 125 : 100)[1].

[1] Our thanks are due to Mr A. O. Allen, M.A., B.Sc., for these measurements.
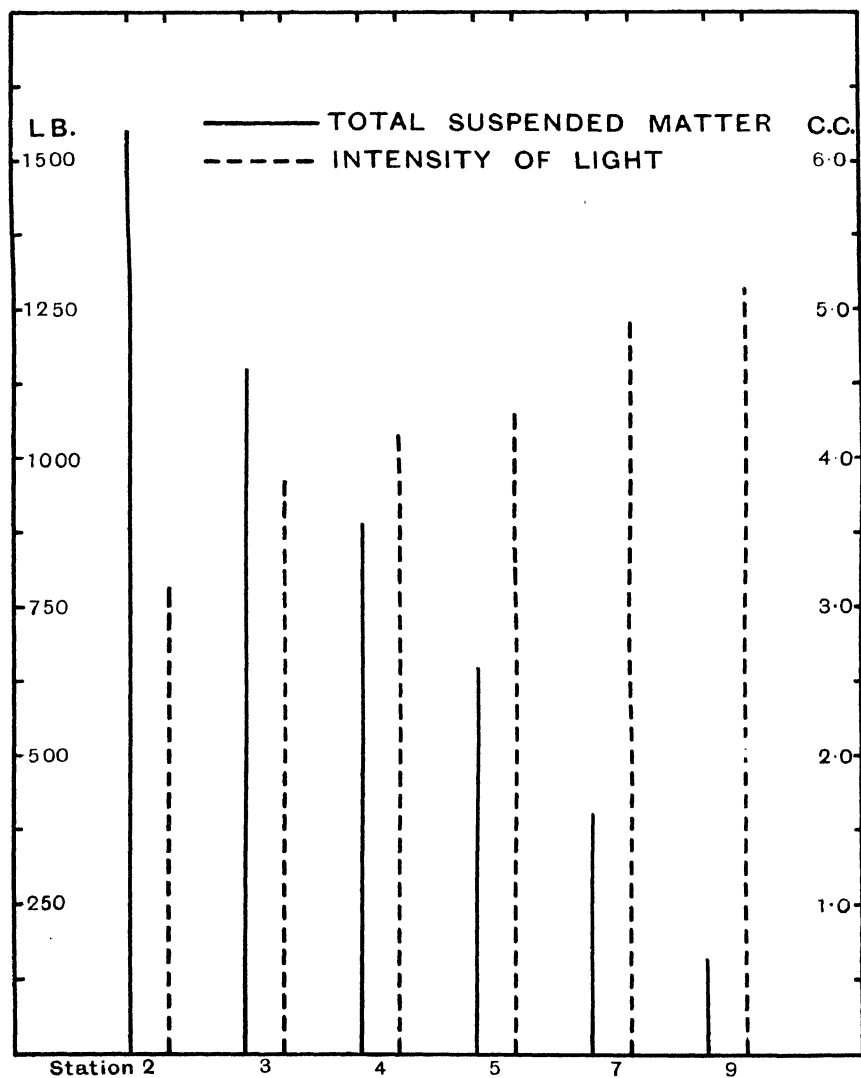
FIG. 3.

Influence of Suspended Atmospheric Impurities upon Intensity of Light.

The effect of all this curtailment of light upon vegetation is only too obvious in the stunted vegetation—such little as survives—of the badly-polluted areas. But the reduced supply of light is by no means the only injurious effect of smoke upon plant growth. The leaves become coated with a thick black deposit which will still further tend to shut out the light and hinder the chlorophyll in its work. The adhesive tarry matter in the deposit will also tend to block up the stomatal openings and thereby impede the free gaseous interchanges which are of such fundamental importance for the growth of plants. The character of these tarry matters is such that once they adhere to the leaves no amount of rain will wash them off. True, the stomata in the case of most plants are situated on the under-side of the leaf, whilst the greater part of the grime will naturally settle upon the upper surface. Still the examination under the microscope of the lower surfaces of the leaves of a variety of plants, showed that they by no means escape unhurt, this being notably the case with evergreens. Most sensitive of all plants to the deleterious influence of smoke would appear to be the conifers. The characteristic sunk stomata of these plants, though serving admirably the purpose of minimising transpiration, form very efficient traps for minute particles of soot when the trees are situated in atmospheres such as prevail to-day in and near our industrial towns. The only two localities in Leeds where conifers attain even moderate growth are those around the two stations which stand out clearly as possessing the purest atmospheres, viz. stations 9 and 10. It is doubtful if they would do well in any district where the suspended impurities deposited yearly amount to 200 lb. per acre.

### *Relative Assimilatory Powers of Leaves from different Localities.*

A number of comparisons have been made of the rate of assimilation of carbon dioxide by cherry-laurel leaves from different parts of Leeds. The results are summarised in Table VII. The sets of leaves to be compared, in each case at least three of the current season's growth, were isolated in flat wooden boxes with glass sides, and air drawn through each box at the rate of about 18 litres per hour for ten hours.

The air after passing over the leaves was deprived of its $CO_2$ by means of sodium hydrate solution, and the amount of $CO_2$ absorbed subsequently arrived at from the amount of standard acid required to make the soda solution neutral to methyl orange after it had been made

neutral to phenolphthalein[1].   By comparison with the result of a
'blank' experiment the nett amount of $CO_2$ assimilated by the leaves
could then be arrived at.

The insolation chambers were fixed outside a window with westerly
exposure, so that they received no direct radiation until late in the day.
The possibility of any disturbing heating effect was thereby reduced to
a minimum.

The temperature of the atmosphere in the vicinity of the chambers
was recorded at short intervals throughout the day.

Measurements were also made of the intensity of illumination at
the place of experiment by the method previously employed (p. 42).

The area of the leaves was measured by means of a planimeter.   In
every experiment leaves taken from the neighbourhood of station 9
were used as 'standard' for the purposes of the comparison.

TABLE VII.

| Experiment No. | Source of leaves | Area of leaves | Intensity of light | Hourly mean temperature | Total $CO_2$ assimilated | $CO_2$ assimilated per 10 sq. in. per 10 hours |
|---|---|---|---|---|---|---|
| | station | sq. ins. | c.c. $\frac{N}{10}$ iodine | degrees C. | milligrams | milligrams |
| 1 | 9 | 36·21 | 1·22 | 10·8 | 21·56 | 5·95 |
| 2 | 9 | 23·5 | 3·4 | 17·6 | 40·48 | 17·2 |
| 3 | 9 9 | 23·52 29·84 | 1·9 | 13.7 | 36·08 37·64 | 11·51 11·82 |
| 4 | 9 5 | 29·84 24·35 | 1·78 | 18·0 | 38·72 16·72 | 12·99 6·86 |
| 5 | 9 7 | 29·84 12·54 | 2·12 | 12·6 | 34·32 6·04 | 11·50 4·81 |
| 6 | 9 5—7 * | 29·84 15·62 | 0·46 | 14·8 | 29·01 2·20 | 9·73 1·48 |
| 7 | 9 4 | 28·64 16·42 | 2·8 | 17·6 | 47·21 2·64 | 13·52 1·56 |

* From garden midway between stations 5 and 7.

Experiments 1—3 were of a preliminary nature to test the reliability
of the method of experiment adopted.   The result of Experiment 3
indicates the degree of concordance that was obtained between the
rates of assimilation by different sets of leaves from the same district.

[1] Brown and Escombe, *Phil. Trans.* 1900, cxciii. 289; Amos, this Journal, i. 322.

The differences in temperature and brightness of daylight on the different days on which the comparisons were made are reflected in the different rates of assimilation of the 'standard' leaves, these ranging from 5·95 mgms. $CO_2$ per 10 sq. in. of leaf surface on the cold and not very bright day on which Experiment 1 was carried out, to 17·2 on the warm and sunny day that followed (Experiment 2).

The laurel plants found in the more polluted areas were all very stunted in size as compared with those growing near station 9, and consequently only medium-sized leaves could be obtained from these districts. The greatly reduced assimilatory powers of these leaves, due to the direct and indirect inhibitory effects of atmospheric impurities, were well brought out in the results of the experiments. Thus if the assimilatory powers of the leaves from station 9 be placed = 100, those of the leaves from the other districts will be as follows :—

Station 5   ...   ...   ...   ... = 53
   „    7   ...   ...   ...   ... = 42
   „    5—7   ...   ...   ... = 15
   „    4   ...   ...   ...   ... = 11½

The great reduction in assimilatory power in passing from the non-industrial district of station 7 to the more polluted areas of stations 4 and 5 is very striking.

In order to test the effect upon the rate of assimilation of the actual solid deposit found upon the leaves from the two last named areas Experiments 6 and 7 were repeated after carefully cleaning the surfaces of the leaves with a dry cloth, the 'standard' leaves being subjected to similar treatment. The results may be summarised as follows :—

Station 9...   ...   ...   ...   ... = 100
   „    5—7   Clean   ...   ...   ... =   24⎫
   „      „    Dirty   ...   ...   ... =   15⎭
   „    4   Clean   ...   ...   ... =   19 ⎫
   „      „    Dirty   ...   ...   ... =   11½⎭

The effect was thus distinctly measurable, although the assimilatory powers of the cleaned leaves still remained far below that of the leaves grown in the purer atmosphere of station 9. The difference is readily accounted for by the actual clogging of the stomatal openings and by the general lowering of the vitality of the plants effected more indirectly by their unhealthy environment.

*Influence of Acid Waters upon Growth of Grass.*

An experiment with Timothy grass has been conducted during the past three seasons with the object of ascertaining the effects of systematic application of waters acidulated with sulphuric acid to the soils in which the grass was growing. The grass was sown on May 12th, 1908, in a series of boxes, each one foot square, filled with soil from a well-mixed heap, and treated exactly alike throughout the experiments with the exception of the watering. The boxes were watered, at rates corresponding to the average Garforth rainfall of 25 ins., with waters containing respectively 1, 2, 4, 8, 16 and 32 parts of sulphuric acid per 100,000. Other similar boxes were watered with Garforth rain-water, with neutralised Garforth rain and with Leeds rain-water respectively. It may be added that the acidity of the Leeds rain ranges from $\frac{1}{2}$ to occasionally 10 parts per 100,000.

In the case of the Leeds rain, and of those waters containing the higher degrees of acidity, germination was distinctly checked and the young shoots quickly acquired a yellowish tinge. The grasses that received the heaviest application of acid (32 parts per 100,000) were killed off in little more than six months and not a trace of vegetation of any kind was visible in the following spring. The watering with water containing 16 parts $H_2SO_4$ per 100,000 proved fatal in little over a year.

The weights and composition of the material grown in each case are recorded in Table VIII. In addition to the data given in this table estimations were also made of total ash, silica, lime, phosphoric acid and potash. The results, however, showed no apparent correlation with the acidities of the waters used.

It will be observed that in every case the yield steadily decreased with increased acidity of the water used and that the effects in this respect were cumulative.

In the first season only the '32 parts' shows any decided falling in yield. In 1909 the 'Garforth Neutral' stands out prominently, the yield being depressed in all other cases. The grass in the '32 parts' box is dead and that in the 16 parts per 100,000 is *in extremis*, the great dividing line now being between the '2 parts' and '4 parts.' In 1910 the differences are still more accentuated; the grass in two of the boxes is dead, in two other cases ('4 parts' and '8 parts') the yield is minimal; even the grass in the '2 parts' box is suffering severely, and, most interesting of all, the Leeds rain is now definitely

telling its tale. The line of demarcation now separates off the ' Leeds Rain ' box and those receiving waters with acidities of 2, 4, 8, 16 and 32 parts per 100,000 from the rest.

TABLE VIII.

| Description of water used | Total dry matter | | | Nitrogen in dry matter | | | Crude fibre in dry matter | | |
|---|---|---|---|---|---|---|---|---|---|
| Year... | 1908 | 1909 | 1910 | 1908 | 1909 | 1910 | 1908 | 1909 | 1910 |
| | gms. | gms. | gms. | °/₀ | °/₀ | °/₀ | °/₀ | °/₀ | °/₀ |
| Garforth rain, neutralised | 28·0 | 24·9 | 14·7 | 2·47 | 2·22 | 1·58 | 24·3 | 21·9 | 23·7 |
| ,,    ,,    ordinary .. | 24·8 | 18·5 | 11·0 | 2·01 | 1·75 | 1·54 | 25·9 | 25·3 | 26·0 |
| Leeds rain ................ | 23·8 | 17·5 | 6·6 | 1·96 | 1·42 | 1·23 | 26·4 | 26·3 | 27·2 |
| 1 part H₂SO₄ per 100,000 | 30·5 | 18·2 | 12·0 | 1·89 | 1·61 | 1·40 | 25·5 | 27·3 | 26·2 |
| 2 parts   ,,    ......... | 28·7 | 17·8 | 8·0 | 1·84 | 1·06 | 1·09 | 26·3 | 28·8 | 28·7 |
| 4   ,,    ,,    ......... | 28·8 | 10·0 | 3·9 | 1·74 | 0·95 | 0·86 | 27·4 | 28·9 | 28·9 |
| 8   ,,    ,,    ......... | 24·8 | 8·2 | 3·7 | 1·77 | 0·89 | 0·82 | 28·2 | 33·4 | 30·3 |
| 16   ,,    ,,    ......... | 23·8 | 1·8 | nil | 1·62 | 0·87 | --- | 30·8 | 36·2 | --- |
| 32   ,,    ,,    ......... | 14·1 | nil | nil | 0·93 | --- | --- | 31·6 | -- | --- |

When the results of the analyses of the grasses are examined it is seen that the increase in acidity besides reducing the total yield of dry matter, lowered the nitrogen-content and increased the content of crude fibre. Here again also the effects are found to be cumulative, particularly in the case of the nitrogen-content. The grass grown with the more acid waters must obviously therefore have been very much inferior in nutritive value to that grown with the neutral or less acid waters. The effect is quite evident in the case even of the grass watered with water containing only one part of acid per 100,000, a proportion which is about the average in Garforth rain, and hence it becomes a matter of the highest interest and importance to ascertain if and to what extent such deterioration can be traced on the large scale in the meadows of smoke-affected areas.

After the removal of the third year's growth, the soils in the different boxes were sampled for the purposes of chemical and bacteriological examination. The results are summarised in Tables IX. and X.

In the chemical investigations, total nitrogen was determined by the Kjeldahl-Gunning method; ammonia by distillation with magnesia under reduced pressure[1]; nitrates by extraction with water and

[1] Russell, this Journal, III. 233.

reduction to ammonia; available phosphoric acid and potash by Dyer's method; total carbonate by Amos' method[1]; and absorptive power for oxygen by the method of Russell[2]. The results in the case of the last named estimation are expressed in terms of the length of the column of mercury which is the visible index of the absorption.

TABLE IX.

*Chemical Analyses of Soils.*

| | Nitrogen | | | 'Available' $P_2O_5$ (Dyer) | 'Available' $K_2O$ (Dyer) | Total carbonate expressed as $CaCO_3$ | Oxygen absorption (Russell and Darbishire) |
|---|---|---|---|---|---|---|---|
| | Total | as Ammonia | as Nitrate | | | | |
| | % | per million | per million | % | % | % | mm. |
| Garforth rain, neutralised... | 0·179 | 3·5 | 5·8 | 0·014 | 0·012 | 0·23 | 12 |
| ,,   ,,   ordinary...... | 0·159 | 1·4 | 4·7 | 0·019 | 0·019 | 0·18 | 9 |
| Leeds rain   ,,   ...... | 0·162 | 2·5 | 3·7 | 0·026 | 0·031 | 0·14 | 5 |
| 1 part $H_2SO_4$ per 100,000... | 0·154 | 2·1 | 4·1 | 0·023 | 0·015 | 0·16 | 8 |
| 2 parts   ,,   ............ | 0·158 | 4·9 | 3·3 | 0·026 | 0·021 | 0·12 | 6 |
| 4   ,,   ,,   ............ | 0·128 | 6·8 | 1·3 | 0 026 | 0·026 | 0·10 | 4 |
| 8   ,,   ,,   ............ | 0·126 | 7·9 | 0·5 | 0·041 | 0·029 | 0·05 | 4 |
| 16   ,,   ,,   ............ | 0·144 | 8·1 | 0·0 | 0·043 | 0·042 | 0·04 | 3 |
| 32   ,,   ,,   ............ | 0·133 | 9·4 | 0·0 | 0·046 | 0·043 | 0·03 | 0 |

With the exception of the total nitrogen a distinct correlation is traceable in all cases between the amount of acid applied to the soil and the different quantities estimated. Thus, increasing acidity has clearly led on the one hand to diminished content of nitrates and of carbonates, and to diminished absorptive power for oxygen, but on the other hand to increased content of the easily soluble mineral ingredients and also of ammonia. The increase in the ammonia-content of the soil with increased applications of acid finds a ready explanation in the results of the bacteriological investigations and will be discussed in connexion with them.

It will be noted further that the effects of the Leeds rain are mainly intermediate in extent between those of the ' 1 part' and ' 2 parts' solutions of sulphuric acid. This is in accordance with the average acidity found in these samples (*vide* Part II.).

[1] Amos, this Journal, I. 322; also Marr, III. 155.
[2] This Journal, I. 261.

## TABLE X.

### *Bacteriological Investigation of Soils.*

| | Total number of Bacteria per gm. of dry soil | Ammonia produced from Peptone | Ammonia converted into Nitrates | Nitrogen Fixed per gm. of Mannite |
|---|---|---|---|---|
| | thousands | mgs. | mgs. | mgs. |
| Garforth rain, neutralised | 5228 | 13·9 | 1·02 | 4·6 |
| ,,      ,,    ordinary... | 1690 | 12·7 | 0·84 | 3·7 |
| Leeds rain      ,,      ... | 1170 | 11·7 | 0·73 | 3·0 |
| 1 part H₂SO₄ per 100,000 | 1260 | 12·1 | 0·80 | 3·3 |
| 2 parts      ,,   ......... | 1100 | 11·2 | 0·85 | 3·0 |
| 4   ,,      ,,   ......... | 690 | 10·5 | 0·52 | 2·8 |
| 8   ,,      ,,   ......... | 130 | 10·3 | 0·36 | 2·4 |
| 16   ,,      ,,   ......... | 40 | 10·3 | 0·28 | 1·9 |
| 32   ,,      ,,   ......... | 15 | 8·1 | 0·13 | 1·8 |

The influence of the treatment with acid upon the extent and character of the bacterial flora of the soil will be seen to have been very striking, the total number of bacteria diminishing rapidly with increasing acidity, this being reflected also in diminished activity as regards ammonia-production, nitrogen-fixation, and, above all, nitrification.

*Total Number of Bacteria.* The data given under this head in Table X. represent the numbers of bacterial colonies that developed upon ordinary gelatine nutrient media. They shew a marked correlation with the crops of grass obtained in the last season (Table VIII.):—

| | Yield of dry matter, 1910 | Colonies per gm. soil |
|---|---|---|
| | gms. | thousands |
| Garforth rain, neutralised...... | 14·7 | 5228 |
| ,,      ,,    ordinary......... | 11·0 | 1690 |
| Leeds rain      ,,      ......... | 6·6 | 1170 |
| 1 part per 100,000............... | 12·0 | 1260 |
| 2 parts      ,,      ............... | 8·0 | 1100 |
| 4   ,,      ,,      ............... | 3·9 | 690 |
| 8   ,,      ,,      ............... | 3·7 | 130 |
| 16   ,,      ,,      ............... | nil | 40 |
| 32   ,,      ,,      ............... | nil | 15 |

The inhibitory effect of even minimal quantities of free mineral acid upon bacterial activity is strikingly exemplified in the case of the two 'Garforth Rain' soils, the numbers of bacteria capable of growing

upon gelatine being reduced to less than one-third by the use of the rain in its natural state with an average content of free acid equivalent to barely 1 part $H_2SO_4$ per 100,000.

The use of the '16 parts' and '32 parts' acid waters is seen to have not only destroyed the grasses, but also to have rendered the soils almost void of living bacteria.

*Ammonia-producing Bacteria.* The activity of these bacteria was measured by their effect upon peptone. The procedure in each case was to incubate $1\frac{1}{2}$ gms. of the soil with 100 c.c. of a nutrient solution containing 1 per cent. of peptone, but no other source of nitrogen. The incubation at $30°$ C. was prolonged for three days and the ammonia produced then estimated by distillation with magnesia. In each case a strong growth was obtained at the surface of the peptone solution, but the rate of development and appearance of the film varied so markedly in the different flasks as to permit almost of a grading of the soils on this basis alone.

It will be noted that whereas ammonia-production was appreciably more active in the 'Garforth Neutral' soil than in those which had received acid waters, the diminution of activity with increasing acidity was very gradual and slight, being only about 25 $°/_o$ less even in the '16 parts' soil. There was, however, a marked falling off in the case of the '32 parts' soil.

*Nitrification.* The most marked effect of the acidity is seen in the reduced activity of the nitrifying or nitrate-producing organisms.

This was investigated in each case by incubating 5 gms. of the soil with 100 c.c. of a sterile nutrient medium containing ·05 gm. of ammonium sulphate, ·5 gm. of calcium carbonate being also added. After 21 days' incubation at $30°$ C. the nitrates formed were estimated colorimetrically by the picrate method. The films obtained were in no case very well developed, which was not surprising in view of the initial poverty of the soil in calcium carbonate. The amounts of nitrate produced were thus in all cases very low, but still the influence of acidity is brought out very sharply. There is again the general concordance between the results obtained with the two ordinary rain-waters and the '1 part' and '2 parts' soils.

It will be noted that the activity of the nitrifying organisms was inhibited to a relatively far greater extent than that of the ammonia-producing organisms. Thus, for instance in the '4 parts' soil whereas the nitrate-production was only one-half, the ammonia-production was fully 75 per cent. of that in the 'Garforth Neutral' soil. This furnishes

a ready explanation of the fact, previously recorded, that the ammonia-content of the soils was greater the greater the amounts of acid that had been applied to them (p. 51).

*Nitrogen-fixing Organisms.* The activity of these organisms was measured by incubating 1 gm. of soil with ·5 gm. of calcium carbonate and 100 c.c. of a sterile nutrient solution containing 2 gms. mannite, ·02 gm. each of monopotassium phosphate, crystallised magnesium sulphate, and sodium chloride, and ·1 gm. of calcium sulphate. After incubation for 13 days at 30° C. the nitrogen-content of the mixture was determined by the Kjeldahl-Gunning method. The amount of nitrogen fixed was then arrived at by deducting the nitrogen originally present in the one gram of soil taken.

The characteristic growth of the nitrogen-fixing organisms has been fully described by Ashby[1] and the sequence of gas-production, growth of film and gradual darkening in colour referred to by him were exactly reproduced in these cultures.

The differences in the rate of development with the different soils were, however, so marked that it was quite possible from the appearance of the cultures to place the soils in substantially the order which was subsequently found to be that of their nitrogen-fixing activities.

Here again, as in the case of nitrification, the application of acid waters to the soil is seen to have exercised a marked inhibitory effect upon the activity of the nitrogen-fixing organisms, the reduction in the case of the soil on which the more impure rain-water (Leeds) was used amounting to practically one-third of the nitrogen-fixing activity of the 'Garforth Neutral' soil.

It is also interesting to note that here again, as in the case of the other forms of bacterial activity investigated, the Leeds rain-water exercises an inhibitory effect somewhat greater than that of water containing one part of $H_2SO_4$ per 100,000 but not greater than that of water containing two parts of $H_2SO_4$ per 100,000. This accords precisely with the indications of the yields of dry matter and with the results of the analyses of Leeds rain samples recorded in Part II.

## CONCLUSIONS.

No claim is made that the investigations recorded in the preceding pages constitute more than a rough survey of the wide area covered by the problem of the influence of atmospheric impurities upon the growth

[1] This Journal, II. 38.

of plants. They do show clearly that this influence, even outside the towns, may under certain conditions have an appreciable bearing upon farm economy. They will, it is hoped, serve as a useful preliminary to a more careful and extended survey of the field.

Attention may be directed specially to the following points brought out clearly by the investigations :—

1. The atmosphere in and around a large industrial city such as Leeds is relatively highly charged with impurities, many of which exert a marked deleterious effect upon plant growth.

2. The impurities are most abundant in the industrial quarters of the city, but are disseminated over very large areas, especially in the direction of the prevailing winds.

3. The rain falling through such a polluted atmosphere becomes notably rich in suspended matters, chlorides, sulphates (often also other sulphur compounds, such as $SO_2$), nitrogenous compounds (notably ammonia) and free acid.

4. The suspended matters in the air impede the growth of plants not only by their deposition upon the leaves and consequent hindrance to the free passage of gases into and out of the leaves, but also by reducing the intensity of illumination of the leaves, such reduction amounting in the worst case investigated to fully 40 per cent. of the maximum light available.

5. The presence of free acid in the atmosphere exercises a detrimental influence upon plant growth :—

(a) By direct action upon the leaves.

(b) Indirectly by reducing the activity not only of the necessary ammoniacal fermentation of the soil humus, but even more of the beneficial nitrifying and nitrogen-fixing organisms in the soil.

6. The experiment with the perennial meadow grass, Timothy, indicates that with the continued application to the soil of acid rain the produce becomes distinctly poorer in protein and richer in crude fibre, and consequently less nutritious. If this holds good for meadow grasses in general it is a matter of great practical importance as bearing upon the nutritive value of meadow hay grown in smoke-infested areas.

In conclusion, we desire to express our appreciation of the valuable assistance rendered to us in the collection of samples and the general conduct of the investigations by a large body of friends. We would specially acknowledge our indebtedness to Prof. J. B. Cohen, F.R.S. for placing at our disposal the information and experience gained in his earlier work upon the subject.

## APPENDIX.

### TABLE A.

*Range of Variation in Composition of Garforth Rain.*

### Parts per million.

| | Number of Samples | Nitrogen | | | | Sulphur | | Chlorine | Free Acid expressed as $H_2SO_4$ |
| | | as Ammonia | as Nitrate | as Albuminoid Ammonia | Total | present as Sulphate $SO_3$ | present in other forms oxidisable to Sulphate $SO_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| Oct. 1906–Dec. 1907 | 83 | Monthly Averages | | | | | | | |
| Minimum ...... | ...... | 0·58 | 0·00 | — | 0·70 | 7·10 | — | 1 39 | 0·71 |
| Maximum...... | ...... | 2·10 | 0·41 | — | 2·51 | 18·09 | — | 10·40 | 6·60 |
| Jan. 1908–Dec. 1908 | 24 | | | | | | | | |
| Minimum ...... | ...... | 0·31 | 0·00 | 0·00 | 0·37 | 7·79 | 0·50 | 0·81 | 0·85 |
| Maximum...... | ...... | 3·20 | 1·61 | 0·72 | 4·14 | 21·32 | 10·25 | 11·30 | 23·10 |
| Jan. 1909–Dec. 1909 | 25 | | | | | | | | |
| Minimum ...... | ...... | 0·32 | 0 07 | 0·09 | 0·49 | 5·46 | 0·00 | 0·98 | 0·48 |
| Maximum...... | ...... | 2·97 | 0·75 | 0·54 | 3·87 | 21·53 | 7·24 | 21·75 | 4·79 |
| | | Individual Samples | | | | | | | |
| Minimum ...... | ...... | 0·22 | 0·00 | 0·00 | 0·33 * | 3·21 | 0·00 | 0·33 | 0·00 |
| Maximum...... | ...... | 4·18 | 3·80 | 1·24 | 7·51 | 29·08 | 14·64 | 21·75 | 41·37 |

Excluding 'Albuminoid Nitrogen.'

TABLE B. *Variation in Composition of Monthly Rain Samples at different Stations in Leeds* (parts per million).

| | | Suspended Matters | | | Nitrogen | | | | Sulphur | | | Chlorine | Free Acid expressed as $H_2SO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | 'Tar' | Ash | present as Ammonia | present as Nitrate | present as 'Albuminoid Ammonia' | Total | present as Sulphate | present in other forms oxidisable to Sulphate | Total | | |
| | | | | | | | | | $SO_3$ | $SO_3$ | $SO_3$ | | |
| **Station 1 (Leeds Forge)** | Maximum | 512·5 | 49·2 | 285·6 | 6·4 | 0·0 | 2·0 | 7·4 | 29·3 | 10·4 | 39·7 | 29·2 | 9·8 |
| | Minimum | 24·6 | 3·5 | 9·0 | 0·7 | 0·0 | 0·0 | 0·9 | 11·2 | 0·0 | 11·2 | 10·8 | 0·0 |
| **Station 2 (Hunslet)** | Maximum | 555·3 | 18·6 | 262·0 | 5·9 | 0·0 | 0·8 | 6·8 | 34·1 | 8·9 | 42·1 | 33·2 | 14·7 |
| | Minimum | 59·0 | 3·2 | 27·7 | 0·5 | 0·0 | 0·0 | 0·9 | 15·1 | 1·4 | 17·2 | 9·1 | 0·0 |
| **Station 3 (Beeston)** | Maximum | 458·2 | 59·3 | 242·5 | 4·9 | 0·4 | 0·8 | 5·6 | 87·2 | 25·3 | 85·1 | 15·8 | 11·7 |
| | Minimum | 68·5 | 11·4 | 86·3 | 0·4 | 0·0 | 0·2 | 0·7 | 17·4 | 3·7 | 21·1 | 9·0 | 0·0 |
| **Station 4 (Philosophical Hall)** | Maximum | 299·2 | 39·8 | 67·0 | 6·0 | 0·7 | 1·3 | 7·3 | 65·5 | 17·7 | 83·2 | 43·2 | 18·8 |
| | Minimum | 45·1 | 6·1 | 16·0 | 0·8 | 0·0 | 0·0 | 1·0 | 16·3 | 2·1 | 26·4 | 4·7 | 0·0 |
| **Station 5 (Headingley)** | Maximum | 270·8 | — | — | 4·1 | 0·8 | — | 5·4 | 33·2 | 11·1 | 42·2 | 24·9 | 3·3 |
| | Minimum | 83·8 | — | — | 1·2 | 0·0 | — | 1·8 | 17·2 | 3·7 | 24·3 | 5·9 | 1·1 |
| **Station 6 (Armley)** | Maximum | 303·9 | 25·6 | 183·8 | 3·6 | 0·4 | 1·8 | 5·4 | 31·8 | 10·9 | 36·7 | 14·1 | 12·7 |
| | Minimum | 24·6 | 1·9 | 13·2 | 0·7 | 0·0 | 0·4 | 1·3 | 13·3 | 1·1 | 17·8 | 8·3 | 0·0 |
| **Station 7 (Woodhouse Moor)** | Maximum | 156·9 | 13·4 | 103·6 | 3·5 | 0·4 | 0·8 | 4·1 | 25·9 | 32·6 | 58·5 | 33·2 | 10·8 |
| | Minimum | 42·1 | 1·3 | 16·6 | 0·2 | 0·0 | 0·1 | 0·6 | 9·4 | 0·9 | 10·3 | 2·5 | 0·0 |
| **Station 8 (Kirkstall)** | Maximum | 176·2 | 13·3 | 94·8 | 2·5 | 0·2 | 1·0 | 3·0 | 25·7 | 9·0 | 33·5 | 8·3 | 4·6 |
| | Minimum | 19·5 | 0·3 | 6·9 | 0·8 | 0·0 | 0·2 | 1·0 | 8·9 | 2·6 | 11·4 | 4·3 | 0·0 |
| **Station 9 (Weetwood Lane)** | Maximum | 81·0 | 8·5 | 21·5 | 9·9 | 0·4 | 2·2 | 12·1 | 30·8 | 8·3 | 38·0 | 19·8 | 7·5 |
| | Minimum | 5·6 | 2·1 | 2·4 | 0·2 | 0·0 | 0·0 | 0·3 | 9·3 | 0·0 | 10·4 | 1·3 | 0·0 |
| **Station 10 (Roundhay)** | Maximum | 30·2 | 8·4 | 12·1 | 1·4 | 0·3 | 0·3 | 2·0 | 18·3 | 4·9 | 23·2 | 9·3 | 0·0 |
| | Minimum | 6·2 | 0·3 | 3·4 | 0·2 | 0·1 | 0·0 | 0·3 | 5·0 | 0·0 | 6·2 | 2·3 | 0·0 |

# ON THE ABSORPTION OF AMMONIA FROM THE ATMOSPHERE.

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THROUGHOUT the whole history of agricultural chemistry attention has been fixed upon the ammonia in the atmosphere as a possible source of the nitrogen of vegetation, and without reviewing the early controversy that centred round Liebig, we may agree that the dispute was largely due to the exaggerated opinions then prevailing as to the amount of ammonia in the atmosphere. The most trustworthy determinations are the long series that have been carried out by Lévy[1] at Montsouris, and they agree with other determinations by Ville[2], Schloesing[3] and Müntz and Aubin[4], showing only from ·01 to ·02 of nitrogen as ammonia per million of air even in Paris, with a still smaller proportion in the air of the country.

This small proportion of ammonia may reach the soil in two ways:

(1)  It is dissolved out by the rain.

(2)  It may be absorbed directly by the soil.

The amount of ammonia brought down by the rain has already been fully discussed by one of us[5]. The total amount brought down per acre per annum appears to be tolerably constant for ordinary country air despite variations in the rainfall, but it rises considerably in the neighbourhood of towns. Observations as yet unpublished indicate that a much smaller total amount of ammonia is brought down by

[1] A. Lévy, *Annuaire de l'Observatoire de Montsouris*, 1901.

[2] G. Ville, *Recherches Experimentales sur la Végétation*, Paris, 1868.

[3] T. Schloesing, *Contributions à l'étude de la Chimie agricole*, Paris, 1888.

[4] Müntz and Aubin, *Rapport sur des Recherches de Chimie appliquée à la science agricole et à la météorologie executées au Pic du Midi*, Paris, 1883.

[5] N. H. J. Miller, *Journ. Agric. Sci.* 1905, I. 280.

the rain falling on the extreme western seaboard of Scotland, where the air may be supposed to have received a minimum of contamination from habitations or contact with the land surface.

There remains the question of whether the soil takes in ammonia by direct absorption from the atmosphere. Of course it is well known that the soil is a very effective absorbent of ammonia, and it might be assumed to be capable of taking away from the air even the very small trace which can be determined therein. It must, however, be borne in mind that the soil itself is a possible source of ammonia. The soil organisms are always producing ammonium compounds, so that a small and fairly constant percentage of ammonium salts is to be found in the soil, representing the transition stage between organic compounds of nitrogen and nitrates. As most soils also contain some calcium carbonate, which by interaction with the ammonium salts would give rise to ammonium carbonate, and ammonium carbonate possesses an appreciable tension of dissociation at soil temperatures, the soil itself must be capable of giving off ammonia to the atmosphere above it. Berthelot[1] enclosed soils with dishes of acid under bell jars and found an appreciable transfer of ammonia from the soil to the acid, and Schneidewind[2] found in the laboratory that soil recently manured with ammonium salts will yield ammonia to a current of air; as will be shown later in the paper such losses can become appreciable under field conditions. It will be safest to assume that the soil possesses a certain ammonia tension, more or less in equilibrium with the air above it, but there are no data upon which to base a conclusion as to whether the gradient is normally from the air to the soil or *vice versa*. To a certain extent the question of the source of the ammonia in the atmosphere is involved in this question. Boussingault[3] was of the opinion that the sea-water contains so much free ammonia that the oceans are the great source of atmospheric ammonia and are in equilibrium with the atmosphere so as to maintain the stock at a fairly constant proportion. From this point of view the sea is the source, and the land both by direct absorption and through the intermediate agency of the rain falling upon the land, is reducing the amount in the atmosphere. On the other hand, many terrestrial sources of ammonia can be specified; the smoke arising from coal fires shows its effect in the increased amount of ammonia in the rain of towns, and all decaying animal and vegetable matter must be contributing some ammonia to

[1] M. P. E. Berthelot, *Chimie végétale et agricole*, i. Paris, 1899.

[2] W. Schneidewind, et al., *Landw. Jahrb.* 1910, **39**. Erg.-bd. iii. 221.

[3] Boussingault, *Agronomie*, Paris, 1860–84, ii. 208.

the air. Volcanic emanations afford another terrestrial source of ammonia, though the numbers of active volcanoes in relation to the total amount of the atmosphere would seem to render such contributions inappreciable.

The question of whether the soil does gain any ammonia from the atmosphere by direct absorption is one of considerable importance in any attempts to construct a balance sheet for the nitrogen received by, and removed from, an experimental plot over any length of time, and the following investigations were initiated with the view of obtaining further light upon the problem. In the first place attempts were made to ascertain if ordinary arable soil either gave up or absorbed any appreciable amount of ammonia when a current of air from the open and therefore containing some ammonia was passed over it. A tube of about 6 ft. long and one inch in diameter was filled with arable soil in a moist condition, and connected with a Reiset absorption column, a meter, and a water pump. Another Reiset column and a second meter was linked up to the same pump and arranged so as to draw approximately the same quantities of air through both. The apparatus was placed in the open a few yards from the laboratory, connexion to the water pump being made by a long metal tube. The result proved entirely negative, no difference could be detected in the amount of ammonia absorbed ; although the pump was allowed to run for many days until from one to two thousand litres of air were drawn over in each experiment. The experiments, however, were conducted on much too small a scale to admit of any conclusions, and as no more efficient pump was available the method was abandoned in favour of the one to be described. Other investigators have attempted to measure the maximum amount of ammonia which might be absorbed from the atmosphere by exposing dishes of acid for a considerable length of time, and then neutralising and distilling over the ammonia for determination. It was decided to repeat these determinations, exposing the dishes under such varying conditions as would give some indication of the source of ammonia. Four pairs of dishes of glazed earthenware 26·5 centimetres in diameter and having a surface of 550 square centimetres were exposed in pairs, one dish at about 5 centimetres from the ground, the upper one at about 115 centimetres. The dishes were about 3 cm. deep and were protected from rain by large sheets of glass fixed at a slight angle above them. One pair of dishes was exposed on the lawn in front of the laboratory ; the situation is open, but there are a few house chimneys at no great distance. Two pairs were exposed at different parts of the

Broadbalk wheat field in a very open situation, especially to the west. Another pair were exposed on the Park grass plots where they are even more remote from houses, but are somewhat sheltered by belts of trees. In each dish 50 c.c. of normal sulphuric acid and 200 c.c. of distilled water were placed, the materials being previously freed from ammonia. As this solution was found to dry up too readily, in the latter experiments for which the figures are given 70 c.c. of glycerol replaced an equal volume of water. The dishes were exposed for a month in each case, whereupon the solutions were washed out and replaced prior to the determination of the absorbed ammonia. The early experiments were carried out by the late Mr F. S. Marr, as were the attempts described above to ascertain the effect of soil upon the ammonia content of the air, but the figures contained in the tables were all obtained since his departure from Rothamsted laboratory.

In a preliminary experiment in which dilute acid alone was placed in the dishes, comparatively high results were obtained, about one milligramme of nitrogen being absorbed per dish per month, which would be equivalent to about ·2 lb. per acre per month. It was found, however, impossible to keep the solutions at all clean ; not only was there a considerable influx of dust, but especially in the lower dishes a number of small flies and other insects were caught by the liquid and very probably furnished some ammonia by hydrolysis of their nitrogen compounds. Accordingly in the later experiments each dish was covered by a sheet of brass wire gauze of 100 meshes to the inch, and though this did not entirely exclude dust it kept out the insects which vitiated the early experiments.

Table I. shows the monthly results obtained for the two years from November 1908 to October 1910, expressed as milligrammes of nitrogen absorbed per dish. As the figures obtained for the two years are consistent and show no large variation from month to month it did not seem necessary to carry the work any further. In the first place it will be seen, with certain exceptions to be discussed later, that the upper dish gives almost always a higher result than the lower one. Very markedly is this the case with the dishes exposed near the laboratory, but the difference is comparatively small with the dishes exposed on the grass land. The greater absorption by the upper dish can be set down to the more frequent renewal of the air above the dish ; it seems more probable that the atmosphere is alike in the two levels, and that the observed differences are brought about by the greater play of air upon the upper dishes.

TABLE I.

*Ammonia Absorption.*

(November 1908 to October 1910.)

| | Broadbalk | | | | Park | | Laboratory | |
|---|---|---|---|---|---|---|---|---|
| | Plot 7 | | Plot 12 | | Upper | Lower | Upper | Lower |
| | Upper | Lower | Upper | Lower | | | | |
| November, 1908 ...... | ·425 | ·475 | ·400 | ·350 | ·475 | ·200 | 1·580 | ·450 |
| December    ,,    ...... | ·425 | ·288 | ·350 | ·263 | ·333 | ·263 | ·650 | ·400 |
| January, 1909........ | ·325 | ·333 | ·313 | ·263 | ·313 | ·175 | ·450 | ·333 |
| February    ,,    ......... | ·313 | ·244 | ·350 | ·275 | ·256 | ·294 | ·450 | ·282 |
| March       ,,    ......... | ·306 | ·300 | ·319 | ·250 | ·269 | ·183 | ·413 | ·325 |
| *April      ,,    ......... | 2·063 | 4·000 | 1·531 | 3·500 | ·325 | ·288 | ·413 | ·338 |
| May         ,,    ......... | ·640 | ·525 | 1·042 | ·656 | ·500 | ·438 | ·600 | ·425 |
| June        ,,    ......... | ·344 | ·350 | ·328 | ·325 | ·375 | ·425 | ·575 | ·400 |
| July        ,,    ......... | ·400 | ·281 | ·460 | ·238 | ·350 | ·300 | ·625 | ·413 |
| August      ,,    ......... | ·550 | ·400 | ·625 | ·375 | ·400 | ·425 | ·650 | ·425 |
| September   ,,    ......... | ·550 | ·450 | ·350 | ·375 | ·400 | ·400 | ·800 | ·525 |
| October     ,,    ......... | ·525 | ·350 | ·350 | ·250 | ·350 | ·325 | 1·313 | ·550 |
| Nov. 1908--Oct. 1909 | 6·866 | 7·996 | 6·418 | 7·120 | 4·346 | 3·716 | 8·519 | 4·866 |
| Average......... | ·572 | ·666 | ·535 | ·523 | ·862 | ·309 | ·709 | ·405 |
| November, 1909 ..... | ·500 | ·367 | ·525 | ·288 | ·545 | ·375 | ·625 | ·450 |
| December    ,,    ...... | ·700 | ·575 | ·525 | ·333 | ·475 | ·317 | 1·833 | ·433 |
| January, 1910........ | ·450 | ·425 | ·450 | ·375 | ·425 | ·350 | 1·313 | ·400 |
| February    ,,    ......... | ·475 | (·575) | ·425 | (·688) | ·525 | ·450 | ·950 | ·525 |
| †March      ,,    ......... | 1·200 | 5·125 | 1·625 | 5·375 | ·475 | ·333 | ·500 | ·325 |
| April       ,,    ......... | ·780 | ·850 | ·800 | 2·000 | ·438 | ·338 | ·475 | ·350 |
| May         ,,    ......... | ·494 | ·481 | ·735 | ·525 | ·425 | ·475 | ·450 | ·250 |
| June        ,,    ......... | ·400 | ·325 | ·288 | ·338 | ·288 | ·650 | ·250 | ·225 |
| July        ,,    ......... | ·475 | ·500 | ·475 | ·450 | ·375 | ·475 | ·525 | ·950 |
| August      ,,    ......... | ·500 | ·396 | ·563 | ·375 | ·459 | ·490 | ·625 | ·646 |
| September   ,,    ......... | ·550 | ·504 | ·587 | ·400 | ·441 | ·373 | 1·175 | ·529 |
| October     ,,    ......... | ·488 | ·775 | ·750 | ·450 | ·375 | ·213 | 1·000 | ·525 |
| Nov. 1909—Oct. 1910 | 7·012 | 10·898 | 7·748 | 11·597 | 5·246 | 4·839 | 9·721 | 5·608 |
| Average......... | ·584 | ·908 | ·646 | ·966 | ·437 | ·403 | ·810 | ·467 |

Manures applied April 7, 1909.        † Manures applied March 16, 1910.

It would be noticed that in certain months of the year the amount absorbed by the dishes above the arable Broadbalk field is much above

the normal, rising from an average of little over ·5 to as much as 5 milligrammes of ammonia per dish. On this occasion also the lower dishes give the higher results. This sudden jump in the amount of ammonia in the atmosphere only occurred over the Broadbalk field, and occurred in April 1909 and in March 1910, months which coincided with the application of ammonium sulphate and chloride as manures to the soil of these fields. The highest results are obtained in the months of application (April 1909 and March 1910) but the quantity absorbed is still much above normal in the following month, especially in 1910, in which year the manure was put on as late in March as the 16th, whereas in the previous year the manuring had been on the 7th of the month. Thus the application of an ammoniacal manure affects the air for more than three but less than seven weeks after its application. As the dishes were removed during the actual sowing of the manures and only replaced in their stands a day or two afterwards, the sudden rise in the amount of ammonia absorbed cannot be due to the intrusion of any manure dust, but must have been brought about by an actual evolution of ammonia from the soil, and this conclusion is strengthened by the fact that the lower dishes nearest to the soil absorbed the most ammonia. The amount absorbed from the two dishes during the month of application and the one following, is in itself comparatively small and would not amount to a pound of nitrogen per acre, whereas about 83 pounds per acre were supplied in the fertiliser.

Small as this amount actually absorbed is, it can only represent a fraction of the total ammonia given up by the soil to the air over this manured field, so that we may fairly conclude that the loss of ammonia as such is by no means negligible when the land is manured with ammonium salts. The Rothamsted soil is only slightly calcareous, containing in this field from two to three per cent. of calcium carbonate in the surface nine inches of soil; as it is also a heavy soil retentive of moisture, the losses would tend to be below the normal of most soils.

Turning now to a comparison of the three situations, Table II shows the total absorption for ten months of each year, excluding April and May 1909 and March and April 1910, the two months following the applications of the manures on Broadbalk.

It would be seen that the arable land gives slightly higher results than the Park grass land, and that both are distinctly below the results on the laboratory lawn. These results are not consistent with an absorption of the ammonia by the soil, because one must suppose that the bare arable soil would have a more powerful absorbing effect upon

the air above it than the grass land.  Moreover, there is no falling off in the absorption of nitrogen above the arable land in the months of September and October, months of the year during which this field (permanent wheat) is being cultivated, when it would expose a maximum of fresh absorbing surface.  The small difference between the grass and arable land is best explained by the difference in exposure, which results in a much more vigorous air circulation above the arable field; and this conclusion is strengthened by the fact that the greater absorption above the arable land is much more marked for the upper dishes than for the lower.

<div align="center">TABLE II.</div>

*Total Nitrogen as Ammonia absorbed per dish in* 10 *months* (mg.).

|           | Broadbalk, Plot 7 | | Broadbalk, Plot 12 | | Park | | Laboratory | |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|
|           | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower |
| 1908 - 1909. | 4·163 | 3·471 | 3·845 | 2·964 | 3·521 | 2·990 | 7·506 | 4·103 |
| 1909--1910... | 5·032 | 4·923 | 5·323 | 4·222 | 4·333 | 4·168 | 8·746 | 4·933 |

The dishes on the laboratory lawn shew by far the highest figures, due doubtless to the greater contamination of the air by the chimneys that are near.  The difference between the upper and lower dishes is also most pronounced on the laboratory lawn, which would again agree with the conclusion that the source of ammonia is aerial, *i.e.* that in the main it comes from the chimneys.

It may be noticed that the amount of absorption by the upper dish on the laboratory lawn reached comparatively high figures on certain occasions, being over the milligramme in November 1908, October and December 1909, and January, September and October 1910.  It has not been found possible to correlate these variations with the prevailing weather, but it should be noted that, with the exception of September 1910, they occur in the winter half of the year when the consumption of coal would be at a maximum.  If the comparison between winter and summer months is made for all the plots, again excluding the two months in each year, Table III., it is only the laboratory station which shows any increase of ammonia during the winter as compared with the summer months.

## TABLE III.

*Average Absorption of Ammonia per month, Winter and Summer*
(excluding April, May, 1909, and March, April, 1910).

(mg. *per dish.*)

| | Broadbalk, Plot 7 | | Broadbalk, Plot 12 | | Park | | Laboratory | |
|---|---|---|---|---|---|---|---|---|
| | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower |
| Winter months, October—March | ·448 | ·428 | ·432 | ·344 | ·395 | ·286 | ·888 | ·425 |
| Summer months, April—September | ·474 | ·410 | ·490 | ·378 | ·390 | ·446 | ·631 | ·485 |

As the rain also derives its ammonia from the atmosphere one might expect there would be some connexion between the richness of the air in ammonia as determined by this absorption method and the amount of ammonia brought down by the rain during the same period. In Table IV a comparison is made between the average monthly absorption in the three dishes situated on Broadbalk field and the Park, and the quantity of ammonia in the rain which is collected in a third field still in the open, though rather nearer to the village of Harpenden than either Broadbalk or the Park.

The concentration of the rain in ammonia depends to some extent upon the magnitude of the rainfall, being higher in months of low rainfall. In consequence the figures are given for both parts of ammonia per million of rain, and total ammonia per acre brought down each month. It will be seen that there is no connexion between the two sets of figures, either inverse or direct.

The results set out in Table I. for the nitrogen absorbed per dish may now be recalculated as lb. per acre and kilos per hectare, Tables V. and VI.

The surprising feature about these results is the small magnitude of the annual absorption per acre. The highest average result for the two years is only 1·533 pounds per acre of nitrogen (1·718 k. per h.) absorbed for the lower dish over the Broadbalk field, the upper dish near the laboratory giving slightly lower figures.

## TABLE IV.

### *Ammonia Absorption and Ammonia in Rain.*

### (November 1908 to October 1910.)

| | Nitrogen absorbed per acre | | Nitrogen in Rain | | Rainfall |
|---|---|---|---|---|---|
| | Upper* | Lower* | Per million | Per acre | |
| | lbs. | lbs. | | lbs. | inches |
| November, 1908 ......... | ·070 | ·055 | ·800 | ·149 | 0·821 |
| December ,, ......... | ·060 | ·044 | ·500 | ·250 | 2·065 |
| January, 1909............ | ·052 | ·042 | ·781 | ·173 | 0·978 |
| February ,, ........... | ·050 | ·044 | 1·281 | ·125 | 0·430 |
| March ,, ........... | ·049 | ·039 | ·470 | ·398 | 3·742 |
| April ,, ........... | ·212 | ·422 | ·450 | ·186 | 1·822 |
| May ,, ........... | ·118 | ·087 | ·766 | ·228 | 1·315 |
| June ,, ........... | ·057 | ·059 | ·400 | ·370 | 4·097 |
| July ,, ........... | ·066 | ·045 | ·425 | ·298 | 3·097 |
| August ,, ........... | ·085 | ·065 | ·719 | ·446 | 2·739 |
| September ,, ........... | ·070 | ·066 | ·917 | ·403 | 1·943 |
| October ,, ........... | ·066 | ·050 | ·263 | ·309 | 5·187 |
| Nov. 1908—Oct. 1909... | ·955 | 1·018 | — | 3·335 | 28·236 |
| November, 1909 .. ...... | ·084 | ·056 | ·550 | ·155 | 1·245 |
| December ,, ........ | ·092 | ·066 | ·250 | ·193 | 3·416 |
| January, 1910............ | ·072 | ·062 | ·350 | ·173 | 2·183 |
| February ,, ........... | ·077 | ·093 | ·225 | ·192 | 3·780 |
| March ,, ........... | ·178 | ·586 | ·300 | ·084 | 1·235 |
| April ,, ........... | ·109 | ·172 | ·625 | ·224 | 1·581 |
| May ,, ........... | ·089 | ·081 | ·450 | ·220 | 2·164 |
| June ,, ........... | ·053 | ·070 | ·475 | ·302 | 2·806 |
| July ,, ........ ...... | ·072 | ·077 | ·425 | ·192 | 1·996 |
| August ,, ........... | ·082 | ·068 | ·400 | ·306 | 3·379 |
| September ,, ........... | ·085 | ·070 | ·525 | ·106 | 9·889 |
| October ,, ........... | ·087 | ·078 | ·475 | ·304 | 2·830 |
| Nov. 1909—Oct. 1910... | 1·080 | 1·479 | — | 2·451 | 27·504 |

\* Mean of Broadbalk and Park.

Kellner[1], at Tokio, who exposed dishes of nearly the same size for two years, got an average absorption of 10·51 pounds per acre (11·78 k. per h.). Heinrich[2] at Rostock by exposing dishes 10 centimetres in diameter for two years, obtained 25·2 pounds per acre or 30·6 k.

[1] O. Kellner, &c., *Landw. Jahrb.* 1886, xv.

[2] R. Heinrich, *Wollny's Forschungen*, 1881, iv. 446.

## TABLE V.

### *Ammonia Absorption.*

#### (November 1908 to October 1910.)

Nitrogen per acre (lb.)

| | Broadbalk | | | | Park | | Laboratory | |
|---|---|---|---|---|---|---|---|---|
| | Plot 7 | | Plot 12 | | Upper | Lower | Upper | Lower |
| | Upper | Lower | Upper | Lower | | | | |
| November, 1908 ...... | ·069 | ·077 | ·065 | ·057 | ·077 | ·032 | ·256 | ·073 |
| December ,, ...... | ·069 | ·047 | ·057 | ·043 | ·054 | ·043 | ·105 | ·065 |
| January, 1909......... | ·052 | ·054 | ·052 | ·043 | ·052 | ·029 | ·073 | ·054 |
| February ,, ........ | ·051 | ·039 | ·057 | ·045 | ·042 | ·047 | ·073 | ·045 |
| March ,, ......... | ·050 | ·049 | ·052 | ·040 | ·044 | ·029 | ·067 | ·052 |
| *April ,, ......... | ·335 | ·649 | ·248 | ·568 | ·052 | ·047 | ·067 | ·054 |
| May ,, ......... | ·104 | ·085 | ·169 | ·106 | ·081 | ·071 | ·097 | ·069 |
| June ,, ......... | ·056 | ·057 | ·054 | ·052 | ·061 | ·069 | ·094 | ·065 |
| July ,, ......... | ·065 | ·016 | ·075 | ·038 | ·057 | ·050 | ·102 | ·067 |
| August ., ........ | ·089 | ·065 | ·102 | ·061 | ·065 | ·069 | ·105 | ·071 |
| September ,, ......... | ·089 | ·073 | ·057 | ·061 | ·065 | ·065 | ·130 | ·085 |
| October ,, ......... | ·085 | ·057 | ·057 | ·040 | ·057 | ·052 | ·214 | ·089 |
| Nov. 1908—Oct. 1909 | 1·114 | 1·298 | 1·045 | 1·154 | ·707 | ·603 | 1·383 | ·789 |
| Average......... | ·093 | ·108 | ·087 | ·096 | ·059 | ·050 | ·115 | ·066 |
| November, 1909 ...... | ·081 | ·060 | ·085 | ·047 | ·085 | ·061 | ·102 | ·073 |
| December ,, ...... | ·113 | ·093 | ·085 | ·054 | ·077 | ·052 | ·297 | ·070 |
| January, 1910......... | ·073 | ·069 | ·073 | ·061 | ·069 | ·057 | ·214 | ·065 |
| February ,, .. ....... | ·077 | ·093 | ·069 | ·112 | ·085 | ·073 | ·154 | ·085 |
| †March ,, ......... | ·195 | ·832 | ·263 | ·872 | ·077 | ·054 | ·081 | ·052 |
| April ,, ......... | ·127 | ·138 | ·129 | ·325 | ·071 | ·054 | ·077 | ·057 |
| May ,, ......... | ·080 | ·079 | ·119 | ·085 | ·069 | ·077 | ·073 | ·040 |
| June ,, ......... | ·065 | ·052 | ·047 | ·054 | ·047 | ·105 | ·040 | ·037 |
| July ,, ......... | ·077 | ·081 | ·077 | ·073 | ·061 | ·077 | ·085 | ·154 |
| August ,, ......... | ·081 | ·064 | ·091 | ·061 | ·074 | ·080 | ·102 | ·104 |
| September ,, ......... | ·089 | ·082 | ·096 | ·065 | ·071 | ·061 | ·191 | ·086 |
| October ,, ......... | ·080 | ·126 | ·121 | ·073 | ·060 | ·035 | ·162 | ·085 |
| Nov. 1909—Oct. 1910 | 1·138 | 1·769 | 1·255 | 1·882 | ·846 | ·786 | 1·578 | ·908 |
| Average......... | ·095 | ·147 | ·105 | ·157 | ·070 | ·065 | ·131 | ·076 |

\* Manures applied April 7, 1909.    † Manures applied March 16, 1910.

per h.   Bineau[1] at Lyons also exposed dishes whose diameter is only
approximately given; taking, however, the quoted figure of 6 centimetres
his result would be 53·6 k. per h. in Lyons and 14·7 at Caluire.
This author states that the Lyons figures were much affected by
dust, and that there were many gnats at Caluire, but as soaking small
gnats in his dilute acid for eight days gave him only half a milligramme
of nitrogen, he concluded that they had not affected the result appreciably.
It is, however, to the dust and living objects which find their way into
the acid that we attribute the discrepancy between our own results and
those of previous observers, and as it is impossible to exclude this source
of error entirely no absolute value can be attached to the results.
Moreover, the wire gauze screen placed over the dishes in our experi-
ments may have a considerable effect upon the rate of absorption, by
checking the rapidity with which the air is renewed above the dish.
To ascertain the effect of the gauze covering two dishes, one covered
with the gauze, the other uncovered, were exposed in the open field for
three weeks in the winter, when it was expected that dust and insects
would be at a minimum.   The following figures show that the exposure
of the uncovered dish was so much greater that six times as much water
evaporated from it and four times as much ammonia was absorbed.
Though the exposure was made in January when the land was pretty
wet a good deal of dust still found its way into the uncovered dish.

In a second trial the covered and uncovered dishes were exposed in
a large unheated building, the doors of which were open from time to
time.   In this case the two dishes absorbed almost the same amount of
ammonia though the evaporation was much greater from the uncovered
dish.

|  |  | Ammonia absorbed mgm. N. | Evaporation, c.c. |
|---|---|---|---|
| Open field—21 days winter | Covered dish | 0·225 | 130 |
|  | Uncovered dish | 1·000 | 790 |
| In-doors—10 days winter | Covered dish | 0·200 | 45 |
|  | Uncovered dish | 0·212 | 115 |

These results indicate that the free play of air over the uncovered
dishes has increased the absorption, the figure obtained for the

[1] A. Bineau, *Études chimiques, sur les eaux pluviales et sur l'atmosphère de Lyon et de quelques points des environs*, Lyon, 1854.

## TABLE VI.

### *Ammonia Absorption.*

### (November 1908 to October 1910.)

Nitrogen per hectare (kilos)

| | Broadbalk | | | | Park | | Laboratory | |
| | Plot 7 | | Plot 12 | | | | | |
| | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower |
|---|---|---|---|---|---|---|---|---|
| November, 1908 ... ... | ·077 | ·086 | ·073 | ·064 | ·086 | ·036 | ·287 | ·082 |
| December ,, ...... | ·077 | ·052 | ·064 | ·048 | ·061 | ·048 | ·118 | ·073 |
| January, 1909... ... .. | ·059 | ·061 | ·057 | ·048 | ·057 | ·032 | ·082 | ·061 |
| February ,, ......... | ·057 | ·014 | ·064 | ·050 | 047 | ·053 | ·082 | ·051 |
| March ,, ....... .. | ·056 | ·055 | ·058 | ·045 | ·049 | ·033 | ·075 | ·059 |
| *April ,, ......... | ·375 | ·727 | ·278 | ·636 | ·059 | ·052 | ·075 | ·061 |
| May ,, ......... | ·116 | ·095 | ·189 | ·119 | ·091 | ·080 | ·109 | ·077 |
| June ,, ... ...... | ·063 | ·064 | ·061 | ·059 | ·068 | ·077 | ·105 | ·073 |
| July ,, ......... | ·073 | ·051 | ·084 | ·043 | ·064 | ·055 | ·114 | ·075 |
| August ,, ......... | ·100 | ·073 | ·111 | ·068 | ·073 | ·077 | ·118 | ·077 |
| September ,, ......... | ·100 | ·082 | ·064 | ·068 | ·073 | ·073 | ·145 | ·095 |
| October ,, ......... | ·095 | ·064 | ·061 | ·045 | ·064 | ·059 | ·239 | ·100 |
| | | | | | | | | |
| Nov. 1908—Oct. 1909 | 1·248 | 1·451 | 1·170 | 1·293 | ·792 | ·675 | 1·549 | ·884 |
| | | | | | | | | |
| Average......... | ·104 | ·121 | ·098 | ·108 | ·066 | ·056 | ·129 | ·074 |
| | | | | | | | | |
| | | | | | | | | |
| November, 1909 ..... | ·091 | ·067 | ·095 | ·052 | ·095 | ·068 | ·114 | ·082 |
| December ,, ...... | ·127 | ·104 | ·095 | ·061 | ·086 | ·058 | ·333 | ·079 |
| January, 1910......... | ·082 | ·077 | ·082 | ·068 | ·077 | ·064 | ·239 | ·073 |
| February ,, ......... | ·086 | ·104 | ·077 | ·125 | ·095 | ·082 | ·173 | ·095 |
| †March ,, ......... | 218 | ·932 | ·295 | ·977 | ·086 | ·061 | ·091 | ·059 |
| April ,, .... .... | ·142 | ·155 | ·145 | ·364 | ·080 | ·061 | ·086 | ·064 |
| May ,, ......... | ·090 | ·088 | ·134 | ·095 | 077 | ·086 | ·082 | ·045 |
| June ,, ......... | ·073 | ·059 | ·052 | ·061 | 052 | ·118 | ·045 | ·041 |
| July ,, ......... | ·086 | ·091 | ·086 | ·082 | ·068 | ·086 | ·095 | ·173 |
| August ,, ......... | ·091 | ·072 | ·102 | ·068 | ·083 | ·089 | ·114 | ·117 |
| September ,, ......... | ·100 | ·092 | ·107 | ·073 | ·080 | ·068 | ·214 | ·096 |
| October ,, ......... | ·089 | ·141 | ·136 | ·082 | ·068 | ·039 | ·182 | ·095 |
| | | | | | | | | |
| Nov. 1909—Oct. 1910 | 1·275 | 1·982 | 1·406 | 2·108 | ·947 | ·880 | 1·768 | 1·019 |
| | | | | | | | | |
| Average......... | ·106 | ·165 | ·117 | ·176 | ·079 | ·073 | ·147 | ·085 |

\* Manures applied April 7, 1909.　　　† Manures applied March 16, 1910.

three weeks exposure in the winter being of the same order as those obtained in our preliminary trials with open dishes. Dust however was not absent and may have been a factor in the higher figure for the

uncovered dish; it is evidently impossible both to exclude dust and permit a quite free play of air. These results render it more probable that the higher results obtained for the upper dishes are due to the increased circulation of air, but before any certainty can be reached it will be necessary to determine whether the air at four feet above the ground does actually contain more ammonia than that down on the surface.

For the same reasons it is hardly possible from these experiments to draw any final conclusion as to whether the soil is an absorber or a source of ammonia. It would be possible to interpret the results on the supposition that the ammonia is added to the air from above, *i.e.* by chimneys, etc., and that the smaller amount absorbed by the lower dishes is due to the reduction in ammonia in the air close to the soil that has been effected by absorption by soil. The greater amount of ammonia in the air near the laboratory would favour this view.

On the other hand it is difficult to suppose that there can be such a difference in the ammonia content of the air of the two levels, considering the freedom of movement of the open air and the rapidity with which the ammonia can diffuse through it. Again the results show on certain occasions the soil of Broadbalk field is giving off ammonia to such an extent as markedly to enrich the layer of air immediately in contact with the soil, and if the soil is palpably giving off ammonia during the months immediately following the ammoniacal manuring, it must still be doing so at other times of the year, for it has been found that there are always some free ammonium salts in the soil of the Broadbalk field, though the amount sinks to a low and comparatively constant level very shortly after the application of manure.

Pending however the determinations of the distribution of ammonia in the air which are now being undertaken it is impossible to come to any more definite conclusion as to whether the soil is absorbing or giving off ammonia.

The main object of the investigation has however been attained and it can be concluded with some confidence that whether the soil normally gives off or absorbs ammonia, the total amount of absorption is very small. Even assuming the soil is as effective an absorbing agent as the sulphuric acid in our experiments the maximum absorption per annum amounts to less than a pound per acre, a quantity that would be negligible in any estimates of the gains and losses of nitrogen on a given piece of land under any method of treatment.

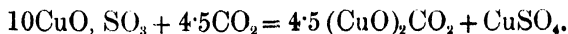# THE ACTION OF CARBON DIOXIDE ON BORDEAUX MIXTURES.

By C. T. GIMINGHAM, F.I.C.

*University of Bristol.*

THE fungicidal properties of the insoluble copper compounds, present in Bordeaux mixtures, have long been known, and it is an important point to ascertain the precise manner in which the poisonous action is exerted. A variety of theories have been put forward, but the matter cannot be regarded as settled. It has been generally accepted that, in order to account for the fungicidal action, it is necessary to look for some means by which the insoluble copper in Bordeaux mixture is rendered soluble when the material is sprayed on to foliage. Atmospheric carbon dioxide has frequently been suggested as the most important agent by which this is effected, and recently this view has been urged by Mr S. U. Pickering, F.R.S., who has also investigated in detail, the chemical nature of the compounds found in Bordeaux mixtures.

Pickering showed[1] that the addition of lime, in gradually increasing amounts, to solutions of copper sulphate, resulted in the formation of a series of basic sulphates of copper. The final compound present in ordinary Bordeaux mixture (*i.e.* made from equal weights of lime and copper sulphate) is $10CuO, SO_3, 3CaO$ in presence of large excess of free lime; in Bordeaux mixtures made without excess of lime, it is either $4CuO, SO_3, 5CuO, SO_3$ or $10CuO, SO_3$, depending on the proportion of lime-water added, the latter being the compound employed in making Woburn Bordeaux Paste.

Pickering then states his opinion that when any of these basic sulphates are sprayed on to foliage they are gradually decomposed by the carbon dioxide of the air with the formation of copper carbonate and some copper sulphate, according to the following equation:

$$10CuO, SO_3 + 4{\cdot}5CO_2 = 4{\cdot}5(CuO)_2CO_2 + CuSO_4.$$

[1] *Journ. Chem. Soc.* 1907, xci. 1988.

To the soluble copper sulphate liberated in this way he attributes the main fungicidal action of Bordeaux mixtures.

A full account of the experiments on which this theory is based are to be found in the *Eleventh Report of the Woburn Experimental Fruit Farm*, 1910, pp. 22—36[1]. A stream of carbon dioxide was passed through Bordeaux mixtures of various compositions and the amount of copper appearing in solution was estimated at intervals varying from 30 minutes after the beginning of the experiment up to 8 days. With the compounds $4CuO, SO_3$ or $10CuO, SO_3$, considerably more copper was dissolved than had been expected from the equation. The excess over the calculated amount is explained by the dissolution of the copper carbonate in the solution of copper sulphate and calcium sulphate formed during the reaction. With ordinary Bordeaux mixture, the amount of copper brought into solution was very small, the excess of lime being attacked by the $CO_2$ much more readily than the basic sulphate of copper. Pickering therefore considers that Bordeaux mixtures made without excess of lime will be considerably more efficient than the ordinary mixture and will have an additional advantage in that there will be no delay in the initiation of the action.

While carrying out some experiments suggested by this work, it was found, most unexpectedly, that the whole of the copper brought into solution by the action of $CO_2$, for a short length of time, on the sulphate $10CuO, SO_3$, was entirely reprecipitated in an insoluble form on removal of the $CO_2$; and the bearing of this reaction on the question of the fungicidal action of Bordeaux mixtures has been investigated.

### *Action of $CO_2$ for a short period.*

The basic copper sulphates, in all the experiments to be mentioned, were prepared by the addition of the necessary amount of pure lime-water to copper sulphate solution and were always obtained precisely as described by Pickering. Carbon dioxide was then passed through the resulting mixture for 30 or 45 minutes, and after filtration the amount of soluble copper present at once determined[2]. On standing exposed to air, the dissolved copper in the filtrate began to reprecipitate almost at once and finally all was thrown out of solution in the form of a light blue scum and deposit.

[1] See also Pickering, *Journ. Agric. Sci.* Vol. III. p. 171.

[2] The potassium ferrocyanide colorimetric method, as detailed in Sutton's *Volumetric Analysis*, 8th. ed. p. 213, was employed throughout and found to give very satisfactory results, the addition of ammonium nitrate to the liquids making the colour a more pronounced pink and rendering the test very sensitive.

The following table gives a few examples of the kind of results obtained:

TABLE I.

| | Per cent. Cu in solution | | |
| --- | --- | --- | --- |
| | Before treatment | After action of $CO_2$ for 30 minutes | After filtrate had stood over-night exposed to air |
| 10CuO, $SO_3$ prepared from— | | | |
| 1. Ordinary lime-water ................ | 0 | ·024 | 0 |
| 2. Pure lime-water ... .................. | 0 | ·022 | 0 |
| 3. Woburn paste .................... ...... | 0 | ·015 | 0 |
| 4. 4CuO, $SO_3$ from pure lime-water | ·0001 | ·022 | * ·00014 |
| 5. 4CuO, $SO_3$   „   „   „ | ·00008 | ·028 | * ·00005 |

* Air passed through filtrate for 2 hours.

### The Basic Sulphate—10CuO, $SO_3$.

When the compound used was 10CuO, $SO_3$ the reprecipitation of the dissolved copper was complete, occasionally in 12–18 hours but more often required 24–48 hours. If, however, a current of air was passed through the liquid, to hasten removal of the carbon dioxide, the whole of the copper came out of solution very rapidly in ½–1 hour. The precipitated compound redissolved readily if carbon dioxide was again passed into the liquid, and while a saturated solution of $CO_2$ was maintained all the copper remained in solution; but as the gas was allowed to escape it began to be precipitated.

The reprecipitation is dependent on the removal of the carbon dioxide from the liquid as is shown by this experiment:

Carbon dioxide was passed into a Bordeaux mixture containing pure 10CuO, $SO_3$ for 25 minutes. There was then 0·02 per cent. Cu in solution. Various portions of this mixture were treated as follows:

Per cent. Cu left in solution.

1. Air blown through for 20 mins. ...      ...      ...      ·00065
2.   „      „      45 „   ...      ...      ...      0
3. After standing for 1 hour in open beaker *with frequent stirring and shaking* ...      ...      ...      ·0042
4. Ditto for 24 hours      ...      ...      ...      ...      0
5. After standing for 1 hour in open beaker *without movement*      ...      ...      ...      ...      ...      ·0155
6. Ditto for 24 hours      ...      ...      ...      ...      ·0072

Similar results were obtained with the filtrate from the mixture.

It seems evident therefore that all the soluble copper in these experiments is in the form of a carbonate dissolved in the $CO_2$ solution. Now, in order to explain this state of affairs it is necessary either to suppose that Pickering's equation for the action of $CO_2$ on the basic sulphates is incorrect and that no copper sulphate as such is formed; or, that the copper sulphate set free according to the equation is removed from solution as it is formed. There is no doubt however that copper sulphate is formed (it can be detected in solution when $4CuO$, $SO_3$ is the compound used); and the latter alternative is the explanation of what happens. Copper sulphate, up to a certain amount, cannot remain in solution in presence of any unchanged $10CuO$, $SO_3$ but combines with it to form a lower insoluble basic sulphate (probably $4CuO$, $SO_3$). This can be shewn to take place directly: *e.g.* in one experiment, $10CuO$, $SO_3$ was prepared from 1 gm. pure $CuSO_4$ and it was found that on gradually adding more $CuSO_4$, this was removed from solution up to 0·19 gm.

In another connexion, Pickering himself refers to this action, though the significance of his statement was not apparent until these experiments had been carried out. On p. 41 of the *Eleventh Report of the Woburn Experimental Fruit Farm*, 1910, he writes: "These basic sulphates may be converted one into another by the addition of either more lime or more copper sulphate....". It therefore appears that, though copper sulphate is formed, it is basic copper carbonate dissolved in the solution of $CO_2$ which constitutes the copper in solution observed in these experiments.

*The Basic Sulphate—$4CuO$, $SO_3$.*

The case cannot be quite the same when $4CuO$, $SO_3$ is treated with carbon dioxide. As it is the lowest basic sulphate prepared by Pickering, any normal copper sulphate set free cannot react with it and so be removed from solution. None the less, all the copper in solution after the action of $CO_2$ for 30 minutes is reprecipitated on blowing air through the filtrate, though not so rapidly as in the case of $10CuO$, $SO_3$. (See Nos. 4 and 5 in Table I.) Soluble copper sulphate is undoubtedly formed since if the compound $4CuO$, $SO_3$ precipitated by lime water and then washed entirely free from calcium sulphate, is submitted to the action of $CO_2$ the filtrate afterwards shews the presence of sulphate, but as soon as all the dissolved copper has been reprecipitated, there is no longer any sulphate found in solution. Evidently therefore the copper sulphate produced in the reaction is

also in this case removed from solution by some means. It appears probable that it combines with the basic carbonate present to form a compound which is precipitated on removal of the $CO_2$; and a reaction between copper sulphate and the basic carbonates of copper, precipitated by $Na_2CO_3$ or $NaHCO_3$, can be shown to take place directly, though not very rapidly.

In one experiment, a solution containing ·015 per cent. copper as copper sulphate was reduced to one of ·0001 per cent. by shaking with a small quantity of washed basic carbonate of copper suspended in $CO_2$-free water. That the soluble copper was not absorbed by the filter paper was shewn by a control experiment. Possibly this may be a case of adsorption.

From these results, it will be seen that whether we start with the compound $4CuO, SO_3$ or with $10CuO, SO_3$, after the action of $CO_2$, the same result is arrived at—any dissolved copper is in a form precipitable on removal of the $CO_2$.

### Action of $CO_2$ for a longer period.

The state of affairs is somewhat different when these basic sulphates are submitted to the *prolonged* action of $CO_2$. Starting with $10CuO$, $SO_3$ which is a full blue bulky precipitate, the action of $CO_2$ for 3–4 hours or longer produces enough carbonate and lower basic sulphate to change the general appearance of the precipitate—it becomes much denser and lighter blue. Under these conditions the copper going into solution is not all easily reprecipitated, particularly if the liquid be removed from contact with the precipitate. A number of experiments have been carried out, but as the conditions (*i.e.* prolonged contact with an atmosphere of $CO_2$) are such that they can hardly affect the question of what occurs in practice, no more than this reference to them is necessary here.

It will be recognised that the results above described, though quite consistent with Pickering's work, are difficult to reconcile with the view that atmospheric carbon dioxide is the important factor in bringing into play the fungicidal properties of Bordeaux mixtures. It was therefore of interest to attempt to test this theory under conditions which approximated more nearly to those actually found in practice.

In the first place, ordinary air was passed through Bordeaux mixtures consisting of $10CuO, SO_3$ or $4CuO, SO_3$ with no excess of lime, for as long as 10 days; at the end of this time no trace of soluble

copper was found in the filtrate. This result has been confirmed a number of times for periods extending from 1 to 5 days. When this experiment is carried out with a very small quantity of $10CuO, SO_3$ suspended in a fairly large bulk of water, the action of the $CO_2$ in the air on the precipitate is indeed quite noticeable—the substance gradually turns a light green colour and becomes much less flocculent, *i.e.* it is largely converted into carbonate, and it shews effervescence with hydrochloric acid. There is evidently sufficient carbon dioxide in the air to convert most of the basic sulphate into carbonate under these circumstances, but not enough to dissolve a detectable amount of the carbonate. In such an experiment the liquid probably contains at least as much carbon dioxide as the film of water present on a leaf under normal conditions.

Again, never more than a very slight trace of copper (equivalent to less than ·00003 gm. Cu) was found in solution when Woburn Bordeaux mixture ($4CuO, SO_3$ or $10CuO, SO_3$) was allowed to stand exposed to air in a shallow layer in a dish for as long as 30 days; and the result was the same whether the film was kept moist with rain water or with ordinary tap water. This is at variance with the result of an experiment of Millardet and Gayon[1] in which a Bordeaux mixture containing no excess of lime exposed to rain on a filter paper showed copper in the filtrate in 7 days, and Schander[2], carrying out a similar experiment, found copper in solution after 21 days. A possible explanation of the discrepancy of these results that suggests itself is connected with the question of filtration. Although the basic sulphates are retained by filter papers comparatively easily, yet when any carbonate has been formed, as would be the case here, it is sometimes a matter of great difficulty to ensure the filtrate being quite free from minute solid particles. From the conditions of Millardet and Gayon's experiment, it seems that incomplete filtration may possibly have accounted for their result.

If a thin layer of precipitated $10CuO, SO_3$ in a Petri dish is exposed to a moist atmosphere of $CO_2$, carbonate is formed and copper is dissolved as would be expected. On then exposing a dish treated in this way to the air for 2 days, only the slightest trace of soluble copper is to be found: that is to say, reprecipitation of any dissolved copper takes place under these conditions in the same manner as in the experiments previously described. This result has been obtained a number of times and it seems safe to conclude that it is only in presence of a large excess of carbon dioxide, such as could hardly occur out-of-doors, that an

[1] *J. d'Agr. Pract.* May 19, 1887.　　　　[2] *Landw. Jahrb.* xxxiii. 1904, p. 517.

appreciable amount of copper is brought into solution from a film of Bordeaux mixture—and that only under those conditions would any copper so dissolved remain in solution.

Most of the experimental work has been carried out with Bordeaux mixtures prepared from pure copper sulphate and pure lime-water, in such amounts that the precipitate should be a definite compound of known composition, either $4CuO, SO_3$ or $10CuO, SO_3$, with no excess of lime present to complicate matters. $10CuO, SO_3$ is the compound present in the commercial Woburn Bordeaux paste, and the paste itself has been used to duplicate the experiments in a number of cases with results which have always been substantially the same as those obtained with the pure preparation.

As Pickering has shewn, the action of carbon dioxide on *ordinary* Bordeaux mixture is practically the same as on the no-excess-lime mixtures except that no copper appears in solution until nearly all the excess of lime has been carbonated. The insoluble compound present in ordinary Bordeaux mixture is $10CuO, SO_3, 3CaO$. If this compound is prepared free from any further excess of lime and is then submitted to the action of $CO_2$, the copper which is rendered soluble is entirely reprecipitated on removal of the $CO_2$, exactly as with the other basic sulphates. It therefore appears that all the arguments based on experiments with the no-excess-lime Bordeaux mixtures are applicable with at least equal force to the case of ordinary Bordeaux mixture.

The conclusion to which these experiments point is that, although the action of carbon dioxide in excess on the compounds present in Bordeaux mixtures brings copper into solution, yet it appears impossible to assign the fungicidal action to copper sulphate liberated by *atmospheric* carbon dioxide. It is difficult to apply experimental results directly to practical conditions because we have no accurate knowledge of the state of affairs in a film of dew or rain on the surface of a leaf; possibly traces of carbonate of copper are present in solution under some conditions; but that there should ever be enough carbon dioxide to dissolve more than very minute amounts seems most unlikely. A purely chemical explanation of the fungicidal action seems insufficient; and in the following paper reasons are put forward for believing that it is probably unnecessary to look for agencies by which the insoluble copper in Bordeaux mixtures is rendered soluble, and that actual contact between the fungus and the particles of the insoluble copper compound is the most important means by which germination or further growth is prevented.

# THE FUNGICIDAL ACTION OF BORDEAUX MIXTURES.

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AND

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THE mixture of copper sulphate and lime universally known as Bordeaux mixture has been in common use as a fungicide in many countries for some time; but it is still uncertain by what means such a mixture, containing no *soluble* copper, is effective. It has undoubtedly a physiological effect upon plants on to which it is sprayed, and may increase their powers of resistance to the attack of parasites; but in addition to this, there appears to be a direct fungicidal action which requires explanation.

It has been generally accepted that the problem resolves itself into discovering in what manner the copper present in an insoluble form is rendered soluble, and a review of the very extensive literature on the subject shews that it is possible to group the various theories as to the fungicidal action of Bordeaux mixtures under three headings :

1. That copper is brought into solution by atmospheric agencies—more especially by the action of the carbon dioxide of the air, *i.e.* a purely chemical explanation.

2. That the leaves, on to which the mixture is sprayed, exert a solvent action on the copper compounds, *i.e.* an action of the host plant.

3. That the fungus itself is responsible for the production of the soluble copper by which it is finally poisoned, *i.e.* an action of the fungus.

It is proposed to discuss the subject in these three divisions.

## I.   *The action of atmospheric agencies.*

The most important work on Bordeaux mixtures from the chemical point of view is by Pickering[1] who showed that the addition of lime to copper sulphate causes the formation of insoluble basic sulphates of copper and not copper hydroxide, as had been previously generally supposed: and from the results of his experiments he concludes that when the mixture is exposed to the air as on a sprayed leaf, soluble copper sulphate is produced by the action of the atmospheric carbon dioxide and forms the active fungicide. This work, however, will not be further referred to here as it is discussed in detail by one of us in the immediately preceding paper, where experiments are described, the results of which, while quite consistent with those of Pickering, appear to make it impossible to accept atmospheric carbon dioxide as the important agent in bringing the fungicidal properties of Bordeaux mixtures into play.

There remains the possibility of a slight solvent action due to rain or dew apart from any carbon dioxide they may contain. Laboratory experiments in which films of Bordeaux mixtures with no excess of lime were exposed to air and kept moist with rain water have given the same results as similar experiments when distilled water is used— no copper appears in solution. The matter is not easy to settle definitely because of the difficulty of eliminating any possible action of the plant itself in practical experiments. Crandall[2] considers that "meteoric waters possess solvent powers that do not belong to water artificially employed" and that "this is probably due to the carbon dioxide and ammonia contained in the rains, but may in part be ascribed to differences in mechanical action, to heavy dews and more complete exposure to the action of the atmosphere." He also finds that ordinary water charged with carbon dioxide has about the same solvent action as natural rain water. These experiments are, however, hardly sufficiently numerous to be conclusive and, as is mentioned later, it is quite possible that some of the results may be accounted for by the solution of some copper by excretions from injured leaves on the experimental trees.

[1] *Journ. Chem. Soc.* xci. 1907, 1988: 11*th Rpt. Woburn Expt. Fruit Farm*, 1910: *Journ. Agric. Sci.* iii. 171.
[2] *Bull.* No. 135, *Univ. of Illinois Agric. Expt. Sta.*

## II.  *The action of the host plant.*

Various writers have devoted attention to the question whether the leaves of plants under normal healthy conditions excrete substances which will dissolve the copper compounds of Bordeaux mixtures : and this is closely connected with a consideration of what is known as Bordeaux injury to foliage.  It can easily be shown that when either leaves or fungus tissues are macerated (or even slightly bruised) in water, the resulting liquid will dissolve these copper compounds readily ; but this does not really affect the question of the actual excretion on to the leaf surface of solvent materials.

Pickering decides against the excretion of any solvent substance from the leaves of most plants under normal conditions and records the following experiment (Eleventh *Woburn Rpt.* p. 109).  Ten large sound leaves of apple, damson and pear were placed in jars of water containing copper carbonate or copper oxide and left for 24 hours with occasional gentle stirring.  Blank experiments without leaves were included and only the faintest traces of copper were found in solution afterwards in all cases.  The authors attempted to repeat this experiment under slightly different conditions, and using Bordeaux mixture precipitates instead of copper carbonate or oxide.

Thus : on June 15, the ends of healthy shoots of gooseberry, currant, apple, and pear, while still on the trees out-of-doors, were fixed in position in jars of distilled water containing either a small quantity of ordinary Bordeaux mixture precipitate (*i.e.* made with equal weights of copper sulphate and lime) or lime-water Bordeaux precipitate (*i.e.* the basic sulphate of formula $10CuOSO_3$ with no excess of lime).  There were three jars of each description to each kind of fruit tree. Injury to the shoots in fixing them in position was avoided.  Controls were included without leaves ; and all were left for 24 hours, after gentle stirring, the jars being loosely covered with paper.  The shoots were then removed, the precipitates allowed to settle, and the clear liquid tested for copper[1] after careful filtration.

In every case in which *ordinary* Bordeaux mixture was used, no copper was to be found in solution.  No doubt under the circumstances of the experiment any copper which might be dissolved was at once repre-cipitated by the large excess of lime present.

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[1] By the ferrocyanide colorimetric method.

The results with the lime-water Bordeaux, which we will call the *no-excess-lime* mixture, were as follows:

| | | | | c.c. standard CuSO$_4$ solution | % copper |
|---|---|---|---|---|---|
| *Controls* (without shoots) | | | | | |
| | 1. | 50 c.c. filtrate | = | nil | nil |
| | 2. | 50 c.c. ,, | = | nil | nil |
| *Gooseberry shoots* | | | | | |
| | 1. | 50 c.c. ,, | = | nil | nil |
| | 2. | 50 c.c. ,, | = | 1·3 | ·00026 |
| | 3. | 50 c.c. ,, | — | 0·7 | ·00014 |
| *Pear shoots*[1] | | | | | |
| | 1. | 25 c.c. ,, | = | 0·6 | ·00024 |
| | 2. | 25 c.c. ,, | — | 0·7 | ·00028 |
| | 3. | 25 c.c. ,, | — | 0·5 | ·0002 |
| *Currant shoots* | | | | | |
| | 1. | 25 c.c. ,, | — | 0·7 | ·00028 |
| | 2. | 25 c.c. ,, | — | trace | ? ·00005 |
| | 3. | 25 c.c. ,, | — | 0·3 | ·00012 |
| *Apple shoots*[1] | | | | | |
| | 1. | 25 c.c. ,, | = | 0·8 | ·00032 |
| | 2. | 25 c.c. ,, | = | 1·0 | ·0004 |
| | 3. | 25 c.c. ,, | = | 1·3 | ·00052 |

This experiment was carried out with similar results on May 19 and September 21. It will be seen that in all cases but one the presence of the leaves brought some copper into solution under these conditions.

Great care was always taken to choose shoots with leaves so far as possible uninjured in any way, but complete freedom from injury could by no means be relied on; consequently these results cannot be used to support the idea of solution of copper by excretions from normal leaf surfaces. Indeed, parallel experiments in the laboratory with detached leaves, under conditions in which closer observation was possible, suggest that the results out-of-doors with the no-excess-lime Bordeaux were due to excretions of organic matter through small injuries to the leaves. Our experiments do not, however, finally settle the question because of the extreme difficulty that was encountered in attempting to pick out a number of leaves entirely free even from minute injuries. There is not the least doubt that very small injuries to the surface of a leaf (punctures by biting insects, etc.) are sufficient to cause some copper to come into solution; indeed definite evidence of this has been obtained in the course of this work. The first small amount dissolved then probably has an injurious effect on the cells in the immediate neighbourhood, which are broken down, their contents then assisting to dissolve more copper, until finally there is enough present to be easily detected.

[1] Shewed signs of Bordeaux injury.

In one experiment, four young leaves of apple were chosen apparently free from any injury, even when examined with a lens. These were suspended in water containing a small quantity of the no-excess-lime Bordeaux mixture, the stalks of the leaves being kept well above the surface. The liquid was gently agitated once or twice and allowed to stand for 24 hours. At the end of this time all the leaves were found to be more or less irregularly spotted (particularly near the edges), the spots being of a much lighter green than the rest of the leaf, usually with the centre brownish; and the liquid contained ·00046 °/$_o$ copper. When the leaves were taken out and examined, it was in most cases possible to discover with a lens the point at which each patch of injured cells began and this strongly suggested an original minute injury of some kind. As the leaves dried, the spots gradually became darker, and finally appeared as dark brown and well-defined areas, quite typical of the scorching action of Bordeaux mixtures.

In other experiments in which the leaves remained longer in the liquids, the amount of copper in solution increased and the injury to the leaves became aggravated. The comparatively large amounts of copper dissolved are no doubt accounted for by the fact that under these special circumstances any solvent excretion from the leaves would be distributed throughout the liquid, and so have its maximum effect.

No scorching action was noticed under the same conditions when ordinary Bordeaux was substituted for the no-excess-lime mixture, evidently because in this case any copper which might be dissolved would at once come in contact with the large excess of lime and be again reprecipitated. This would not necessarily be so under practical conditions when the particles of which the Bordeaux mixture consists are distributed in a thin film over the leaf surface.

Crandall's results[1] are of considerable interest in this connexion. He determined the amount of dissolved copper in the drip water collected from trees sprayed with ordinary Bordeaux mixture and afterwards treated in various ways. Some were protected from atmospheric moisture by means of canvas coverings and were sprayed with "cistern" water or with water charged with carbon dioxide (carbonated water), so as to compare the results with those from similar trees exposed to rain and dew.

It was found that copper appeared more rapidly in the drip water from trees exposed to atmospheric moisture than from those to

which "cistern" water was artificially applied; and the results with carbonated water were about the same as those from exposed trees. The differences in the amounts of soluble copper found in the drip waters are however small, and variations in the degree of injury to the foliage of the various trees may have had some bearing on the results.

Crandall includes the following in his summary:

"Bordeaux mixture on foliage yields soluble copper more rapidly under the action of meteoric waters than under the action of waters artificially applied. Injury to foliage follows the action of rain in some cases, but does not result from water artificially applied.

"The physical condition of leaves at the time of spraying is important. Leaves injured by insects, or attacked by fungi, are especially susceptible to additional injury by Bordeaux mixture."

Then on p. 233 of his paper, discussing the fact that young leaves seem to be less susceptible to injury than older ones, he explains this by their "greater freedom from abrasions that allow penetration of the copper as it becomes soluble." Is it not more probable that excretions from the injuries first actually cause the copper to become dissolved? And, further, may it not be that the less rapid solution of the copper from the trees receiving water artificially applied was partly due to the fact that the foliage of these trees was covered and protected from any damaging action of the wind and presumably to some extent from insects, and so was less injured than that of the exposed trees?

The results discussed here are not indeed conclusive. They do not shew that all scorching caused by Bordeaux mixtures would be avoided if all leaves were free from any previous injuries, and the possibility of a slight solvent action due to uninjured leaves is not settled; but our experience inclines us to lay considerable stress on the part played by material exuded from mechanical injuries to the surface of the leaves in dissolving the copper in Bordeaux mixtures, and such dissolved copper may act poisonously on both leaf and fungus cells. The leaves of fruit trees under ordinary conditions are certainly not for the most part free from small abrasions of one sort or another, particularly from the middle of the summer onwards; and a practical point that lends considerable support to the view here expressed is the greater amount of injury which is noticed following Bordeaux spraying late in the season when compared with the results of earlier applications at a time when the young foliage is in a thoroughly healthy and more or less undamaged condition.

### III.    *The action of the fungus.*

A number of writers have from time to time called attention to the possibility of direct action between the fungus and the insoluble Bordeaux precipitate to account for the fungicidal properties of this spray fluid.    Rumm[1] failed to demonstate the occurrence of solvent excretions from *Puccinia* uredospores, and found that they could germinate in drops of Bordeaux mixture, provided that they were not actually in contact with particles of the copper compound.

Frank and Krüger[2] discovered that, while spores of certain *Perono-sporaceae* germinated in the filtrate of several weeks old Bordeaux mixture, they were killed in the unfiltered liquid.    They also found that spores of *Phoma Betae* germinated well in the filtrate of one-week-old Bordeaux mixture[3].

Swingle[4], in a review of the earlier work, pointed to the possible solvent action of the fungus as one means of explaining the fungicidal properties of the spray fluid.

Aderhold[5] investigated the subject more fully.    He observed that conidia of *Fusicladium pirinum* were able to germinate in exposed drops of the filtrate of Bordeaux mixture, and also in drops of the unfiltered liquid if they were not in contact with particles of the copper compound, so long as the excess of lime was not too great.    He also noticed that they germinated on pear leaves sprayed with the fungicide; but he believed that no germination occurred on the flecks of the Bordeaux precipitate.    Drop cultures of the conidia in rain water collected from sprayed foliage showed free germination : and he concluded that germination could therefore occur under natural conditions in raindrops on the living foliage.    He also dried drops of Bordeaux mixture and arranged strips of filter paper, infected with the conidia and soaked in rain water, with one end resting on the dried drop to allow of the diffusion of soluble copper compounds.    Normal germination occurred at a distance of 10—13 mm. from the drop, but at intervening points the germinations were fewer and more abnormal in character according to the distance from the drop, and at 0—4 mm. no germination took place.    His general conclusion was that Bordeaux mixture hinders but does not absolutely prevent germination : and

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[1] *Ber. deut. bot. Gesell.* Bd. XI. 1893, p. 79 and *Beitr. z. wiss. Bot.* Bd. I. p. 81.
[2] *Arb. d. deut. landw. Gesell.* 1894, Heft 2.
[3] *Ber. deut. bot. Gesell.* 1894, p. 8.
[4] *U.S. Dept. Agr. Div. Veg. Phys. and Path. Bull.* 9, 1896.
[5] *Cent. für Bakt.* Abth. 2, Bd. V. 1899.

therefore its main effect should be regarded as due either (1) to increased fungicidal action on the infection tubes of the fungus after the carbonation of the calcium hydrate, or (2) to the increased resistance powers of the host.

Clark[1] studied the effect of a large number of copper compounds upon the spores of various fungi, and found that on the whole there was a remarkable similarity in the "copper resistance" powers of different species: but his conclusions on this point have since been challenged by Pickering[2]. He favoured Swingle's hypothesis of the solvent action of the fungus, having observed that spores of various fungi placed in water containing "copper hydrate" in suspension, were killed in a short time. The rapidity of killing depended upon the nature of the spore coats. Traces of soluble copper compounds were found after the addition of a large quantity of spores. He asserted that the quantity of soluble copper necessary for the destruction of the spores is probably not more than one part in 80,000 parts of water (dew).

Schander[3] stated that on the basis of his experiments he did not believe that sufficient soluble copper appeared on the leaf to kill fungus spores; and he agreed with Clark that in the main the fungi themselves dissolved as much copper as is necessary to kill them.

Crandall[4] held that no direct evidence has been forthcoming to demonstrate solvent action on the part of the fungus.

Pickering[5] has also arrived at this conclusion for the ordinary case. After leaving the oidia from mildewed apple twigs for 24 hours in water containing copper carbonate in suspension, he found only ·00003 per cent. of soluble copper, whilst in the control experiment without the spores the copper found was ·00004 per cent. He gave, however, no information as to whether the spores were alive or dead at the end of the time, a point of material importance in view of the results described below.

It is thus evident from this survey of previous work on the subject that the question of the existence of a solvent action of the fungus upon Bordeaux precipitates cannot be considered as conclusively settled.

A number of experiments have been made by us with the object of gaining further information upon the subject. The following may be referred to as providing fresh evidence.

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[1] *Bot. Gaz.* 33, No. 1, 1902.

[2] *Eleventh Rpt. Woburn Expt. Fruit Farm*, 1910.

[3] *Landw. Jahrb.* xxxiii. 517, 1904.

[4] *Univ. Illinois Agric. Expt. Sta. Bull.* 135, 1909.          [5] *loc. cit.*

An aqueous solution of dextrose, containing 1 per cent. of this substance, to which a quantity of basic copper sulphate, insoluble in the solution, had been added, was infected with spores of *Penicillium glaucum*. No nitrogenous material was used in this culture fluid in order to avoid dissolving any of the basic copper sulphate. A slight growth of the fungus took place. After 10 days from the time of inoculation the liquid was filtered and tested for soluble copper compounds. The result showed the presence of ·0011 per cent. of copper. A similar solution, uninoculated and used as a control, contained only ·00055 per cent. of copper. The presence of the fungus was therefore responsible for dissolving twice as much copper as was present in solution in the control fluid. While, however, this result may be taken as possible evidence of the existence of a solvent action on the part of the fungus, it must not be overlooked that the fungus was growing under unfavourable conditions and that the solution of the insoluble copper compound may have been effected, not by a solvent normally excreted by the fungus, but by substances diffusing from dead or dying cells of the mycelium in its unhealthy condition. In another case a similar solution of dextrose, to which no copper compound had been added, was infected with *Penicillium* spores : and, after fair growth had occurred, it was filtered carefully and the filtrate shaken up with some basic copper sulphate. This mixture on analysis showed the presence of ·0075 per cent. of soluble copper : while a corresponding control solution, which had not been infected with *Penicillium*, contained no more than ·0004 per cent. of soluble copper. The same criticism, however, may be applied to this as to the previous case. The dextrose solution after the growth of the fungus showed a distinct acid reaction, which doubtless accounted for the solution of the copper compound. Even if it could be claimed that these experiments demonstrated the secretion of a solvent by *Penicillium*, it remains to be proved that other fungi also behave in the same way.

A number of hanging-drop cultures have been made with Bordeaux mixture and the filtrate of Bordeaux mixture, both freshly made and several weeks old. (Except where stated to the contrary the no-excess-lime mixture, containing the basic sulphate $10\,CuOSO_3$, was used.) The behaviour of conidia of *Nectria ditissima*, uredospores of *Puccinia hieracea*, and conidia of the *Monilia* stage of *Sclerotinia fructigena*, has been tested in these. On the whole little difference in the results with the freshly prepared liquids and those several weeks old was observed.

In the drops of Bordeaux mixture infected with *Nectria* conidia as

a rule little or no germination occurred. The large majority of the spores died in the course of a few days. In working with this fungus it was easy to determine whether or not death had taken place, since the conidia are colourless, thin-walled, multicellular structures, and the appearance of the cell contents of living and dead cells is quite distinctive. In many cases some of the cells of a conidium died within 24 hours, the rest remaining alive, although dormant, sometimes for several days. In other cases every cell of a conidium died within a day or two. In rare cases the entire conidium remained living for a time, and after three or four days developed more or less feeble germ-tubes. More often, however, where germination occurred, a portion of the conidium had already died, the germ-tubes being produced from the one or two cells which had not been killed. With rare exceptions the germ-tubes showed very weak growth and were curiously abnormal in form, eventually dying in a few days. In drops of the filtrate a few conidia generally germinated within 24 hours : and after two days these shewed fair growth, while several of the remainder had also germinated. Growth continued until the organism was exhausted from lack of food. In control drops of water better germination and growth in the first 24 hours were recorded.

Conidia of the *Monilia* stage of *Sclerotinia fructigena* were subjected to similar tests. In drops of the Bordeaux filtrate the number of germinations was moderately good, and the subsequent growth was fairly vigorous. When Bordeaux mixture itself was used the conidia rarely germinated : and, even when this occurred, the total growth was very small and weak and the organism soon died. In the same set of cultures conidia situated in small condensed drops of water on the cover-slip adjoining the main drop of Bordeaux mixture germinated well and grew vigorously. The control drops of water showed good germination and strong growth.

When uredospores of *Puccinia hieracea* were used, in the control drops of water and in the Bordeaux filtrate, the number of germinations was large and the growth for a time rapid and strong. In the drops of Bordeaux mixture several spores germinated within 24 hours, although the percentage of germinations was not so high as in the preceding cases. The growth was at first fairly vigorous. No conclusions could be drawn in the case of these cultures as to the cause of the arrest of growth, since the fungus cannot be cultivated as a saprophyte : and death might therefore have been due quite as much to starvation as to any injurious copper effect. It is interesting to record, however, that, unlike the two

other fungi used, both germinations and growth were comparatively free in the presence of the insoluble copper compound. It may at the same time be pointed out that the normal rate of germination and growth of the *Puccinia* is more rapid than that of the other fungi.

Corresponding experiments with this *Puccinia* were made in watch-glasses filled with the respective fluids. The results were similar to those of the hanging-drop cultures.

In all of the above cases the no-excess-lime Bordeaux was used. Ordinary Bordeaux (with excess of lime) was also tested similarly with *Nectria* and *Sclerotinia* conidia. Where the filtrate was used, germination in each case was fairly free and the growth vigorous: but in the drops of the unfiltered mixture, germination was very rare and the subsequent growth very weak. In the latter drops, most of the spores were killed quickly, but some were still alive at the end of four days.

In another set of experiments, deposits of the freshly made Bordeaux precipitate on pieces of filter paper and porous porcelain were used. A film of this substance sufficient to coat well the filter paper and porous porcelain respectively, was obtained by pouring over them a few drops of the unfiltered mixture: and after the surplus liquid had drained away the films were heavily infected with *Nectria* and *Sclerotinia* conidia and placed in a moist atmosphere. One series was also placed is a closed chamber in the presence of caustic soda solution, so that any possible effect of carbon dioxide should be eliminated. The behaviour of the spores was observed by examining microscopically small samples of the films daily. In all cases the results were alike, very few germinations occurring, and the very feeble growth which took place on such occasions soon being killed. Some of the *Nectria* conidia were not entirely killed after four days, although invariably the majority of the constituent cells were dead. The dead germ-tubes of the few *Sclerotinia* conidia which germinated shewed a characteristic yellowing of the terminal protoplasm and an exudation of yellowish brown droplets at their tips, which strongly suggested combination with the copper. The fungicidal action of the Bordeaux compound was evidently not in any way dependent upon the presence of carbon dioxide.

The whole of these results tend strongly to support the hypothesis that certain fungi at least are able to act on the insoluble copper compound sufficiently to poison themselves, provided that they are associated closely enough with it: for in all cases copper was practically absent from the filtrate before use, so that it was obviously the presence of the

insoluble compound which was necessary to give the mixture fungicidal properties. There appear to be only two explanations to satisfy the results. Either the fungus spore in its dormant condition or in the incipient stages of germination exerts a solvent action on the copper compound : or the minute traces of that substance which can be present in a state of solution act as a cumulative poison. An attempt to obtain further light was made by utilising diffusion tubes to prevent direct contact between the copper particles and the spores, while still allowing possible diffusible excretions of the latter to reach the copper. About 10 c.c. of freshly made Bordeaux were placed within the tubes, and these were placed in slightly larger test-tubes containing about 5 c.c. of the filtrate of the same mixture. In some cases the liquid in the outer tube only was inoculated with *Nectria* conidia, while in other instances the Bordeaux in the inner tube was also inoculated. In every case the conidia germinated freely outside the diffusion tube and developed germ-tubes of considerable length. After the previous experiments it was surprising, however, to find that several germinations occurred also in the inner tubes, where these were inoculated. Not only did such germinations take place, but the germ-tubes grew well, like those outside, and remained alive for the whole of the week during which the experiments were under observation. Although these results appear to clash with those obtained with the hanging-drop cultures of Bordeaux mixture, there is not necessarily any serious antagonism between them : and they probably serve very materially to assist in finding an explanation of the action of this fungicide. An examination shewed that the copper precipitate had completely settled on the sides and base of the diffusion tubes, leaving the liquid clear and apparently free from suspended particles. As far as could be ascertained by careful examination of the contents of the tubes, the living and germinated spores occurred only in suspension in the liquid, while the dead conidia were situated in the deposit of the copper compound. These experiments point therefore to the improbability of the fungus exerting any serious solvent action at a distance from the copper, and also to the insufficiency of the cumulative action hypothesis. They suggest, however, that the question of distance between the copper particles and the fungus is the critical matter. In the hanging-drop cultures the conidia and the copper precipitate are in close touch : and the total bulk of the culture is small, so that the solvent action of the fungus—which, if any, must be weak—could occur under concentrated conditions. In the diffusion tube cultures, on the other hand, the

volume of the liquid is very much greater, and many of the spores are comparatively far removed from the copper compound. The solvent action must therefore be seriously reduced, and probably absolutely negligible, except for such spores as are in the immediate vicinity of the copper particles.

The final experiments in this direction seem to establish definitely the correctness of this line of argument. A large number of films of the Bordeaux precipitate on cover-slips were made by allowing drops of the freshly made mixture placed upon them to evaporate. They were put aside for a few hours to dry thoroughly. Drops of distilled water infected with *Nectria* conidia were then carefully placed on these films, so that the edges of the drop overlapped the edge of the film. Every effort not to displace any of the dried particles of the copper deposit was made. Some of these drops were allowed to dry, so that the spores in their turn formed a film superimposed on that of the copper compound and overlapping it. These cover-slip preparations were then placed in a moist atmosphere. The remainder of the drops were not dried off, but were made into the ordinary hanging-drop cultures. After 24 hours marked differences in the respective sets of cultures were found. In the dried set of films kept in a moist atmosphere, there was a sharp limit between regions of living and dead conidia which coincided practically exactly with the edge of the copper film. The conidia above this film were entirely killed, the protoplasm of the cells being shrunken and characteristic; and no remains of rudimentary germ-tubes were observed. Beyond the copper zone nearly all the conidia were living, and had germinated in many cases, especially in the region farthest removed from the copper. In the hanging-drop cultures, on the other hand, the large majority of the conidia, especially those in the lower part of the drop and therefore remote from the copper film, were living and germinated. A few in the upper part of the drop, obviously touching the copper film on the under side of the cover-slip, were either entirely or partially dead.

As far as the thin-walled conidia of *Nectria* are concerned, then, it may be considered as established that actual contact with particles of the insoluble copper compound is sufficient to cause death, although growth can actually occur in the immediate vicinity provided that the organism does not touch the copper. It seems reasonable to postulate, therefore, a solvent action on the part of the fungus which is not powerful enough normally to produce serious toxic effect except when direct contact occurs. At the same time it is evident that such action

is purely local and cannot result in the bringing into solution of sufficient copper to exercise a general fungicidal action over the whole surface of a leaf. Failure to demonstrate the presence of soluble copper in liquids containing simply the basic copper sulphate or carbonate and the fungus spores cannot be held to prove the absence of a solvent action on the part of the fungus. It does not appear to be possible to explain the results stated above unless the solvent power exists, since the experiments with the filtrates shew that there is not sufficient soluble copper originally present to prevent germination and growth.

The results with the *Puccinia* spores serve to throw additional light on the subject. In this case the spore coat is a comparatively thick resistant structure of very different character from the thin delicate coat of the *Nectria* conidium and the typical wall of an actively growing germ-tube. It is probable that, like the cuticle of the upper surface of most leaves, it is more or less impenetrable to substances in solution, whether contained in an external liquid or within the spore itself. Consequently in its dormant state it is not likely to have much effect on or be affected by any copper particles with which it may come in contact. It may therefore begin to germinate in a more or less uninjured condition: and the fate of the delicate germ-tube will then depend on its relation to the particles of the copper compound. If it escapes direct contact there seems to be no reason why growth should not continue, if the behaviour is analogous to that of the *Nectria*: and the germination and growth observed in the various experiments with this fungus are probably to be accounted for in this way, since the Bordeaux precipitate had time to settle in the drop and watch-glass cultures before germination began.

A direct action on the part of the fungus in the manner just outlined, therefore, seems certain: and all the results obtained by previous workers, who have actually studied the behaviour of fungi in the presence of Bordeaux compounds, can be explained without difficulty according to this view. The following explanation of the action is strongly suggested by the observed facts. The cell-wall may be regarded as the critical factor. Considering the case of an isolated cell, an interchange of material between the cell and the surrounding medium is always proceeding, and the passage takes place through the cell-wall. The nature and amount of the material which is thus transferred, while undoubtedly governed by the selective action of the parietal layer of protoplasm, is at the same time regulated by the character of the wall. If the latter is in the condition common to actively growing and

assimilating cells, it is readily permeable by a variety of substances in solution and the rate of interchange is rapid. If, on the other hand, the primary nature of the wall has been altered by the deposition of various substances which render it more or less impermeable by liquids, the character and quantity of the substances which can be transferred are radically changed. The interchange of material through the resistant walls typical of many resting spores—and also through the strongly cuticularised walls of the upper epidermis of most leaves—is probably reduced to a minimum. Consequently in such cases the passage from the cell of substances capable of exercising a solvent action on the insoluble copper compounds of Bordeaux mixture is extremely limited and presumably inappreciable: and conversely the entry of soluble copper into the cell is also difficult. The typical primary unchanged cell-wall, however, being readily permeable, must always be more or less saturated with aqueous solutions containing such of the cell products as are allowed to pass through the parietal protoplasmic layer. It is not unreasonable to suppose—in view of the general character of diffusible plant products—that the wall saturated with a solution of such substances is capable of exerting a solvent action on insoluble particles of the copper compounds of Bordeaux mixtures with which it may come into actual contact: and at the same time the absorption of the dissolved copper through the wall doubtless proceeds rapidly and exerts a toxic action on the living cell contents. Since the extent of the chemical action of the cell secretion in such cases must follow the law of inverse squares, and the same must apply to the absorption of the dissolved copper, it is evident that the distance of the copper particles from the living cell is a matter of primary importance where toxic effects are concerned.

### General Considerations and Summary.

In reviewing this work as to the manner in which Bordeaux mixture is of service as a spray fluid, the distinctions in its possible methods of action, as pointed out by Pickering[1], require consideration. A direct and immediate toxic action on the fungus already present and an indirect protective action on the foliage, rendering it more or less immune from subsequent infection, are both possible alternatives: and to some extent each may occur. In fact, it appears probable that the value of the mixture may be attributed not so much to one particular

[1] *Eleventh Rept. Woburn Expt. Fruit Farm*, 1910.

effect as to a number of distinct modes of influence, varying in importance.

With regard to the direct toxic influence on fungi which have already attacked the host plant the general trend of opinion is that the action in this direction is comparatively limited. Massee[1] has drawn attention to cases where fungi, which have already obtained a hold on the host before the application of the spray, continue to develope on the foliage after spraying; and has mentioned other examples where, after spraying, fungi grew freely on the surface of the Bordeaux deposit. Both he and Salmon, also on the same occasion, emphasize the necessity of regarding Bordeaux mixture as a preventive rather than a direct fungicide. The cases quoted by Massee can be understood in the light of the results recorded above. Where leaves are already infected, only the superficial portions of the fungus can be directly injured: and the extent of such injury depends not only upon the proximity of the particles of the copper compound but also upon the character of the exposed cell-walls of the organism. The development of the fungus on the surface of the Bordeaux deposit appears to depend upon its relative rates of growth and destruction. The whole of the organism is not necessarily in actual contact with copper particles, and the free segments of the mycelium may grow freely if other conditions are favourable, while the parts in contact are killed. Spores may germinate in such positions if the spore coat is sufficiently resistant and the germ-tube is produced on the side of the spore not in contact with the copper. It may be pointed out, in passing, that while such cases are capable of simple explanation if the view of the interaction of the Bordeaux deposit and the fungus, as put forward above, is accepted, it is difficult to account for them by the hypothesis of the production of soluble copper by atmospheric action, since all sections of the organism within reach of the film of moisture on the leaf should then feel the toxic effects.

The indirect influence of Bordeaux mixture by protecting the foliage from further attack is generally regarded as the most important: and this view is hardly likely to be seriously disputed. Whether or not it is due, in part at any rate, to an increase in the resistance power of the host, as suggested by Rumm, Frank and Krüger, etc., has not been conclusively demonstrated: and on this question the present work has no bearing. Other workers, such as Schander and Clark, incline to the view that the protection is afforded by the injurious effect of the copper

[1] "Rept. of Conference on the Spraying of Fruit Trees," *Journ. Roy. Hort. Soc.* Vol. xxxiv. Pt. iii.

on the spores which reach the foliage, either in their dormant condition
or in the incipient stages of germination, this effect being produced by
the solvent action of the fungus on the copper in its insoluble condition.
Crandall, Pickering, and others, have sought in other directions for an
explanation, since the presence of soluble copper as a result of the
action of the fungus has not been conclusively demonstrated : but, as
already pointed out, it is hardly likely that an appreciable amount,
other than that absorbed directly by the fungus, would be brought into
solution.

In view of the evidence brought forward in this paper it is
difficult to see how the existence of a solvent action by the fungus can
be denied : and Pickering, although holding the view that no demonstra-
tion of its existence had been satisfactorily made, seems tacitly to
assume its occurrence, when he refers to the probable ability of the
film of copper carbonate eventually formed on the leaf after spraying, to
inhibit the germination of such spores as may alight upon particles of
the copper deposit. At the same time, if the efficiency of Bordeaux
mixture is to be attributed entirely to this local action between in-
dividual spores, dormant or germinating, and the copper deposit, it
must be admitted that the protection afforded to the host plant is
governed by the distribution of the insoluble copper compound upon it :
and since this must necessarily be more or less incomplete and irregular,
a certain amount of infection must always be liable to occur. The
results of practical experience are not antagonistic to this view : and
it may even be claimed that they favour it more strongly than any
other hypotheses which have been suggested to explain the action of
Bordeaux. At times considerable infection does occur after spraying :
and it is generally recognised that inefficient spraying is comparatively
valueless, and that the more complete the distribution of the fungicide
the better are the results. All practical authorities insist upon the
importance of covering the foliage and the other parts of the plant
liable to attack as thoroughly as possible with a coat of the Bordeaux
precipitate : and it is for this reason that the use of a special Bordeaux
nozzle on the spraying machine is considered imperative. The value of
a second spraying is well known : and this is in many cases given within a
month after the first spraying, while the foliage still bears a considerable
copper deposit from the original application. If the necessity for a com-
plete coating of the foliage is admitted, the advantage derived from the
second spraying is easily understood : but its benefit is not so obvious,
if the production of soluble copper by atmospheric carbon dioxide is

regarded as the means of Bordeaux action, since, if the plant is properly sprayed in the first place and there still remains on the foliage a certain amount of the copper deposit, the excess of lime added in the second spraying will delay the action for a further period, i.e. until most of it is carbonated. Whether or not this direct interaction between the copper deposit in its undissolved state and the fungus is sufficient to account entirely for the beneficial results of Bordeaux spraying, is an open question.

The protective action of the copper film in this manner may be supplemented by the action of copper brought into a state of solution by various agencies. The work of Millardet and Gayon and of Crandall with the drip water of trees, sprayed under practical conditions, appears to establish conclusively the presence of some soluble copper. A portion of this may result from purely chemical changes as distinct from those which may be due to biological factors, such as the action of the plant itself or of the fungus; but the evidence which has been discussed with regard to the influence of leaf injuries seems to indicate that there is a considerable amount at least of the total soluble copper which owes its origin to the latter cause. Crandall himself refers to the dark colour of the drip water: and Pickering admits that such dissolved copper must be at least partly produced by the action of organic substances brought into play as a result of leaf injury. Its fungicidal value, if it is in a condition of electro-negative combination, as appears probable, is, however, uncertain.

It seems unlikely, after reviewing all available evidence, that the plant, except in special cases, has any appreciable effect, apart from the leaf injury action, in the production of soluble copper. This conclusion might be anticipated after the considerations which have been urged in the discussion on the action of the fungus as to the influence of the character of the cell-walls. The structure of the epidermal tissues of the upper surface of the leaves, with the strongly developed cuticle, is such that little direct action by mere contact is probable.

Many workers, such as Millardet and Gayon, Crandall, and Pickering, appear to have approached the problem with the assumption that soluble copper must be present in the film of moisture which more or less intermittently covers the plant, if adequate fungicidal action is to occur: and those named regard its production as due, mainly at any rate, to purely chemical agencies. There is no occasion to consider at length the nature of the copper which might be brought into solution. The likely compounds are the sulphate and one or more of the

basic carbonates, dissolved by water containing carbon dioxide. As far as the copper sulphate hypothesis is concerned, the reasons for its non-acceptance have been already stated from the chemical point of view[1]: and there are also possible objections from the practical side. Any copper sulphate produced on the leaf, which was not washed off at once by rain, would be presumably in a state of solution, the strength of which varied from extreme dilution to extreme concentration according to the degree of wetness and dryness of the leaf. Possibly even, when the leaf was dry, it might be deposited in solid form. Under such circumstances severe leaf injury, far more extensive than that commonly met with in practice, would be expected : and its absence is evidence against the copper sulphate hypothesis.

A dissolved carbonate may possibly be present on occasions : but it is very doubtful if its quantity is sufficient to be of serious fungicidal value. The total quantities of soluble copper found by Crandall in the collected drip water of sprayed trees were very small, and a considerable percentage at least should probably be attributed to organic compounds. It cannot therefore be considered conclusively demonstrated that copper brought into solution by chemical agencies plays any active part in the fungicidal action of Bordeaux mixture.

These results are not without bearing on the practical side of Bordeaux spraying. In the first place they emphasize the necessity for *thorough* spraying, so as to coat as completely as possible the surfaces liable to infection. Secondly, they show that it is important to use the copper precipitate in a very finely divided form, so that the foliage may be more completely covered. Thirdly, they explain how Bordeaux mixture may be effective in action immediately after application : and that this is so, is the opinion expressed by both Clark and Crandall in their papers and generally held by practical experts. Pickering, however, holds the contrary opinion, but quotes no evidence or confirmation of his view. Whether or not any conclusion can be drawn as to the effect of excess of lime in the mixture is rather uncertain. It would appear that, *ceteris paribus*, the greater the excess of lime the less effective is the coating on the leaves, since the copper particles are more widely separated; but the question is evidently complicated by a number of other factors. Finally, the results direct attention to the importance of the adhesive properties of the spray.

There is nothing unfamiliar in the above conclusions; their significance has already been recognised from the results obtained in practice.

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[1] See this vol., preceding paper.

# SOUTH AFRICAN CEREAL RUSTS, WITH OBSERVATIONS ON THE PROBLEM OF BREEDING RUST-RESISTANT WHEATS.

By I. B. POLE EVANS, M.A. (Cantab.), B.Sc., F.L.S.

HISTORY has little of note to relate regarding cereal rusts in South Africa. G. W. Thompson (1) in his *Travels and Adventures in South Africa* informs us that the wheat crops during 1708—1710 were severely attacked by rust. Theal (2) nevertheless maintains that rust in wheat was not known in the country before the year 1727, when the epidemic was so bad that the crops were almost a complete failure, although he states that rust in rye had been known before 1727. The year 1820, however, is reported as one notorious for the severity of the rust attack. The history of the oat crop is of considerable interest, for there is very strong evidence to the effect that rust in oats was not known before 1858, though since that time the crops have never practically been free. These remarks refer especially to the crops in the oldest agricultural colony, the Cape, yet farmers in the Transvaal have repeatedly assured me that when oats were first grown in these more northern parts no such thing as rust was known. To-day it is practically impossible to raise as a summer crop the old and most favoured variety—the Boer Oat—so severely does it suffer from rust. The same thing regarding rust in oats has been experienced in Natal. In former years large crops of oats were raised, whereas now their cultivation has almost been abandoned on account of rust. Although rust has played such havoc with South African cereals for close on two centuries it is somewhat surprising to find how little attention has been paid to this important subject either by scientists or governments of South Africa. It is true that much has been written on the subject in various South African agricultural publications, but most of this relates to information that has been gleaned from investigations abroad. No definite attempt has been made to study the rust-organisms themselves,—the cause of all this

enormous loss,—but it has tacitly been taken for granted that the solution of the problem lay in the discovery of a rust-proof wheat, either by chance introduction from oversea or by cross-breeding of local promising varieties. It is true that the former method has done much to give heavier yields than would have otherwise been obtained, while no work worthy of mention has been performed in the latter branch of research. Since cereals which are rust-resistant in other countries, *e.g.* America, Australia, England and India, do not retain this quality when introduced into South Africa, it will readily be seen that the problem is essentially one of South African importance and requires to be studied in the country before any means to its solution can be expected.

The observations recorded in this paper are only those extending over a period of four years, and are chiefly confined to a very limited area, viz. the most important wheat growing districts of the Transvaal, though frequent field observations have been made from time to time on cereals in various parts of South Africa.

The varieties of rust noted up to the present are :—

    (1)   *Puccinia graminis*, Pers.   The Black rust on :
                    (*a*)   Wheat.
                    (*b*)   Barley.
                    (*c*)   Oats.
                    (*d*)   Rye.
    (2)   *Puccinia triticina*, Eriks.   The Brown rust on :
                    (*a*)   Wheat.
    (3)   *Puccinia coronifera*, Klebahn.   The Yellow rust on :
                    (*a*)   Oats.
    (4)   *Puccinia dispersa*, Eriks.   The Brown rust on :
                    (*a*)   Rye.

*Puccinia graminis*, Pers., the Black rust is the most serious of these rusts. It is very rarely absent from any crop of summer cereals. In the Low Country with its almost tropical climate it is no uncommon thing to find this rust in its uredo stage all the year round. Far from being prevalent and most injurious during extremely wet weather, my observations go to show that it thrives best and causes most damage during hot dry spells, when it becomes so abundant that frequently the soil beneath affected plants is coloured a rusty brown with the uredospores. As a rule the rust is most conspicuous on the lower portion of the stalks of the plants, and from thence gradually spreads to the leaves and ears. *Puccinia graminis* usually first appears on wheat, then on arley, oats and rye in succession. It is also found on *Dactylis glomerata*,

*Lolium temulentum* and *Festuca elatior*. In South Africa no aecidial form of *Puccinia graminis* is known, but only the uredo and teleuto stage. The part played by the teleutospores in the life-history of the rust has yet to be determined, for all attempts to get them to germinate have so far failed.

### Inoculation experiments with Puccinia graminis.

Eriksson (3) from the results of his inoculation experiments in Europe found that the rust on rye readily infected rye and barley, while the rust on barley infected barley and rye. No infection of wheats or oats resulted. The form on wheat sometimes infected barley feebly, rye and oats very rarely. The rust on oats only infected oats and none of the other cereals.

In America Carleton (4) showed that *P. graminis* on wheat readily infected barley and the rust from barley infected wheat, while no infection of oats resulted. The rust on oats also failed to infect wheat, barley or rye, while *P. graminis* on rye infects barley but not wheat. In India the rust from wheat does not readily infect barley, nor does the rust from barley succeed when sown on wheat.

In South Africa my inoculation experiments show that three forms of *Puccinia graminis* on cereals may be distinguished.

(1) The form on rye presents points of considerable interest. Rye in the Transvaal is never attacked until late in the season and not before the plants are in ear. Up to the present it has only been observed on the stalks and not on the leaves. This rust infects barley but not wheat or oats. All attempts to infect rye-leaves with this rust have so far failed. This can only be regarded as a remarkable case of parasitism where a rust growing on the sheath stalk is unable to live on the leaf.

(2) The form of *P. graminis* which occurs on wheat infects wheat and barley but neither rye nor oats. In the field during the winter months barley remains free from rust, while wheat growing alongside may be badly rusted. Here we are again face to face with a striking case of parasitism, viz. of a plant being immune to rust during the winter months but highly susceptible in the summer.

(3) The form of *P. graminis* which is found on oats does not infect wheat or barley. Some hundreds of inoculations have been carried out with this rust and occasionally an infection on wheat or barley has resulted. Seeing that the spore material for these experiments has in nearly all cases been collected in the open where all three crops were

under cultivation and badly rusted, it is quite possible to conceive that the inoculation material used was not as pure as it would have been, had it been taken from plants raised as pure cultures.

It should be mentioned here that although no such thing as rust in oats is known in India, Indian oats when grown in the Transvaal are highly susceptible to both species of rust, *P. graminis* and *P. coronifera*.

### *The problem of breeding rust-resistant wheats.*

With the rediscovery of Mendel's principles of heredity, the wheat rust problem has to-day practically resolved itself into an attempt to breed or produce plants of exceptionally high rust-resisting qualities. In short the whole tendency has been to concentrate attention on the wheat plant itself. In how far this is justified or in how far it has produced the results expected remains yet to be seen. My own observations and experiments so far carried out in South Africa point rather to the fact that this line of investigation is not one that is likely to lead to results of much practical importance so long as the parasites themselves are ignored. These remarks apply especially to a country like South Africa, where as yet we are in complete ignorance of the life-history of any of our cereal rusts.

On reviewing the literature connected with the wheat rust problem for the last twenty or thirty years several points of interest may be noted. For instance we find certain varieties of wheat recommended for their rust-resisting qualities holding good for several years in succession, and then they disappear from view altogether. So far as I am aware no reason for this has been advanced, but there is little doubt I think that it is nothing more than that these varieties have in course of time all fallen prey to the rust pest. It is the deeper reason for this falling off in immunity that we have to seek. Is it a falling off in immunity or an advance in the parasitic properties of the parasite that we are concerned with ? It is only a closer study of the relation between host and parasite that is likely to solve the question.

Farrer (5) who did a vast amount of work on improving and hybridising Australian wheats, which was originally undertaken with the object of making or securing rust-resisting varieties, himself acknowledges after ten years' experimental work that the progress which he had made towards the solution of the rust problem was disappointing.

Biffen (6) in England has now been working at the problem for ten

years. Biffen began his investigations in 1900. The primary object
which he then had in view was the improvement of English grown
wheat. The re-discovery of Mendel's principles of heredity in 1901 led
him to examine the behaviour of possible characters of wheat on
hybridising. Amongst other things he found that immunity and
susceptibility to the attacks of Yellow rust (*Puccinia glumarum*) were
definite Mendelian characters. On crossing a very susceptible variety
with one practically immune to rust, Biffen found that the resulting
hybrid was as rusty as the susceptible parent, but these cross-breeds
produced plants which were rusty and clean in the proportion of
practically 3 : 1. From this it has been concluded that susceptibility to
Yellow rust is a dominant and transferable character, while immunity
is recessive.

This has been considered one of the most important discoveries to
which the Mendelian method has yet been applied, and results in
promise unsurpassed have been expected therefrom. Be this as it may,
we have to pause and ask ourselves have any results of a definite
progressive nature in the physiology of rust-resistance been yet
obtained ? Has any wheat of high rust-resisting qualities yet been
placed upon the markets and which has stood the test of time ?

For the past four years I have paid attention to the behaviour of
the Black rust (*Puccinia graminis*) from the Mendelian point of view,
and although the work on several occasions has been seriously interfered
with by locusts and floods, results of some considerable interest have
been attained. The work has been much on the same lines as that
conducted by Biffen in England, but I have especially confined myself
to a study of the parasite and its relation to its host. For this purpose
a large number of varieties of wheat which showed marked immunity to
rust were crossed with highly susceptible varieties. The wheats most
extensively used in these experiments were Bobs Rust Proof, White
Egyptian, Wol Koren and Holstrooi. Other varieties were also crossed,
but as most of the experiments were carried out on the above varieties
I shall confine myself to them for the present. Bobs Rust Proof and
White Egyptian were selected as the immune parents, Wol Koren and
Holstrooi as the susceptible types. These wheats were chosen because
both field and laboratory experiments and observations in Pretoria had
afforded ample proof of their rust-resisting and non-rust-resisting
qualities respectively. Bobs Rust Proof wheat is one of Australian
origin and was specially recommended by Farrer as being highly
resistant to *Puccinia graminis*. At Pretoria in field experiments and

also in my laboratory this wheat proved exceptionally immune to
*P. graminis,* although in the Low Country and in several other parts
of the Transvaal it was badly attacked with this rust. Although this
wheat was so immune to Black rust (*P. graminis*) it proved both in
field and laboratory experiments to be highly susceptible to the Brown
rust (*P. triticina*). Of the other wheats White Egyptian is a variety
obtained from Egypt and has proved highly resistant to *P. graminis*;
Wol Koren and Holstrooi are local wheats which have been grown in
the country for years and are extremely susceptible. I shall select a few
typical inoculation experiments for illustration.

*Experiment 239.*

Eight plants of the immune variety (Bobs Rust Proof), eight plants
of the susceptible variety (Wol Koren) and eight plants of the hybrid
between these two wheats were raised in separate pots under as similar
conditions as possible, and were then infected with uredospores from
the same source (Klein Koren Wheat). Seven days after inoculation
a number of white flecks were visible in the leaves of the immune
variety (Bobs Rust Proof). The leaves of the susceptible variety (Wol
Koren) were covered with incipient pustules, while the inoculated
leaves of the hybrid were of a deep yellow colour at the inoculated
spots and were apparently full of incipient pustules. On examining the
plants two days later, the immune ones showed 71 small and weakly
pustules, each of which was surrounded by a very discoloured yellow
area. The susceptible plants showed 595 strong pustules which ran
one in another and made it very difficult to count them accurately.
The hybrid plants bore 638 vigorous pustules which were surrounded
by a slightly discoloured area.

This experiment was repeated again under exactly the same condi-
tions, only that 10 plants in each case were used. It gave the following
results. After 12 days:

Immune      plants bore   12 feeble pustules.
Susceptible     ,,      ,,    206 strong     ,,
Hybrid         ,,      ,,    597 vigorous   ,,

After 20 days:

Immune plants showed 114 small pustules.

Susceptible plants bore pustules too crowded together and too
    numerous to count accurately.

Hybrid plants showed 4675 pustules.

Similar results were obtained from inoculating other immune and susceptible parents and their hybrids. These experiments showed clearly that the hybrid resulting from a cross between an immune and susceptible plant, becomes even more susceptible to the Black rust (*P. graminis*) than the susceptible parent. These results were also fully confirmed in the row cultures of these wheats exposed to natural infection at the Botanical Experiment Station, Pretoria. Although the hybrids were plants of more vigorous growth than either of the parents, a careful comparison showed that they were more severely attacked by rust than the susceptible parent. This marked susceptibility of the hybrid was perhaps even more conspicuous on comparing the harvested wheats, when the hybrids could be picked out at a glance owing to the abundance of black teleutospore pustules on the stalks. This extreme susceptibility to rust of the hybrid between immune and susceptible parents does not appear to be peculiar to Black rust (*P. graminis*) for much the same phenomenon occurred in Biffen's trials with the Yellow rust (*P. glumarum*) although little attention appears to have been directed to this remarkable fact. For instance, Biffen states that from six hybrid plants from Michigan Bronze and Rivet wheat highly susceptible and immune parents respectively only three grains were obtained and these failed to germinate, so severe was the attack of rust. Another point worthy of mention and connected with the rust raised on the hybrid wheats in my pot-culture experiments in the greenhouse, is that the majority of these wheats produced abundant crops of teleutospores which rarely ever occurred on either of the parents which were growing alongside and which were similarly treated. The teleutospore formation generally began at the lowest portion of the stalk and spread gradually upwards. The only explanation which I can offer for this extreme susceptibility to rust of the hybrid plants is the fact that being much more vigorous in growth than either of the parents, they afford a larger food supply to the rusts.

The rust borne by these hybrid plants was next investigated in detail, and experiments were planned to determine whether its parasitic properties had in any way become intensified by its sojourn in these hosts. With this object in view a number of inoculation experiments were carried out with rust from the hybrid and susceptible plants respectively. On submitting the parent plants to these rusts, it was found that the rust from the hybrid now readily infected the immune parent. In other words the hybrid acts as an intermediary in the passage of the rust from a susceptible to an immune parent.

Further experiments showed clearly that the rust from the hybrid produced a far more severe infection in both parents than did the rust from the susceptible parent. The following experiment (No. 250) will serve to illustrate this point:—nine susceptible plants (Wol Koren) were inoculated with rust from susceptible plants (Wol Koren) and nine similar plants with rust from the hybrid obtained from Wol Koren and Bobs Rust Proof. In a similar manner nine immune plants (Bobs) were inoculated with rust from the susceptible variety (Wol Koren), while nine others were inoculated with rust from the hybrid. After thirteen days the susceptible plants (Wol Koren) inoculated from the susceptible (Wol Koren) showed 101 pustules, while those inoculated from the hybrid showed 833 pustules.

The immune plants (Bobs) inoculated with rust from the susceptible parent (Wol Koren) showed no pustules, while those inoculated with rust from the hybrid showed 209 pustules. After eighteen days the susceptible plants (Wol Koren) inoculated from the susceptible parent (Wol Koren) showed 432 pustules, while those inoculated from the hybrid showed 1669 pustules. The immune plants (Bobs) inoculated with rust from the susceptible parent (Wol Koren) now showed 82 minute pustules, while those inoculated with rust from the hybrid showed 688 fair sized pustules.

The result of these inoculations shows definitely that the rust from the hybrid is not only able to infect successfully the immune parent, but also that it produces a severer infection on the susceptible parent than rust from the parent itself.

These experiments were then carried a step further. The rust on the susceptible and immune varieties contracted from the susceptible and hybrid plants was next compared as to its powers of infection. For this purpose immune and susceptible plants raised under as similar conditions as possible were inoculated with rust from susceptible and immune plants infected from susceptible and hybrid plants. To quote one experiment (No. 255) in which in each case five plants were used and thus 40 plants in all were inoculated. After 14 days the result was as follows:—the rust from the susceptible plant and which was originally contracted from the susceptible variety had produced 18 minute pustules on the immune plants and 123 small pustules on the susceptible plants, whereas the rust from the immune plants originally contracted from the susceptible variety had produced 62 minute pustules on the immune plants and 294 fair sized pustules on the susceptible variety. In like manner the rust from the susceptible plants originally infected from

the hybrids had produced 226 minute pustules on the immune plants and 971 medium-sized pustules on the susceptible plants, whereas the rust from the immune plants originally infected from the hybrid
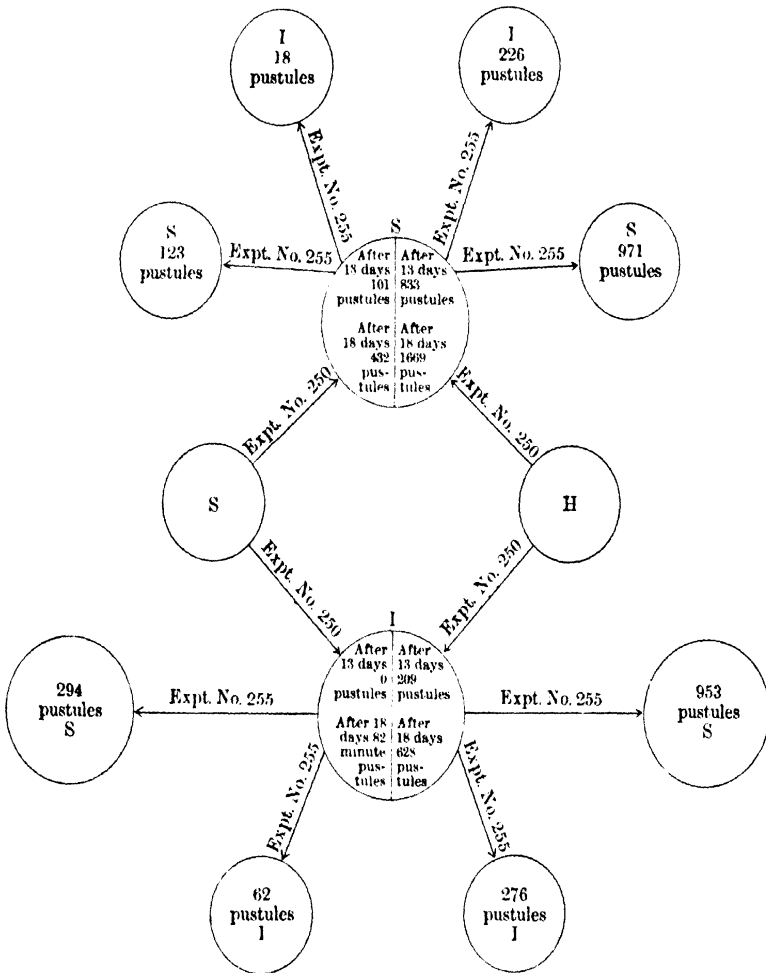


In the above diagram, I denotes immune, S susceptible, and H hybrid plants.

had produced 276 minute pustules on the immune plants and 953 medium-sized pustules on the susceptible plants.

From this experiment it is easy to see that the rust which passes through a hybrid plant produces a far more severe infection than the rust from the susceptible parent. In other words the pathogenic properties of the rust have become distinctly increased after its sojourn in a favourable host. Whether this is simply due to the fungus being in a more vigorous condition for attack, or whether there are more subtle reasons for its increased pathogenic properties, remains yet to be proved, but the fact remains that such hybrid plants may play a very important part in the transmission of rust organisms from susceptible to immune varieties. In this connection it is interesting to note that as far back as 1903 Marshall Ward (7) from the result of his rust infection experiments with the Bromes, in which certain species were successfully infected with spores from plants which usually failed to infect such hosts, says " it seems impossible to avoid the conclusion that we have here to do with species which act *as intermediaries in the passage of the fungus from one section of the genus Bromus (Serrafalcus) to another (Stenobromus).*" " If this existence of 'bridging' species turns out to be well established, we may have a very satisfactory explanation to several important matters ; and if—a far more hypothetical conjecture—we could show that these 'bridging' species are either hybrids or varieties which link one set of predisposed species to others, the importance of their discovery would be greatly enhanced."

If it turns out to be the general rule that these hybrids act in the manner above stated the problem of producing rust-resistant strains will be no easy one, and I think that such hybrids may possibly explain several obscure points connected with immunity.

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# REVIEWS.

**The Physiology of Reproduction.** By F. H. A. MARSHALL, M.A., D.Sc. [Longmans.] This is a comprehensive treatise dealing in an exhaustive manner with the structure and functions of all the organs concerned in reproduction, and with the physiology of all phases of the reproductive processes. It is written with exceptional clearness, the illustrations are excellent and much to the point, and full references are given to the literature of the subject.

Some few years ago Mr Walter Heape in his book on the breeding industry drew attention to the economic importance of the study of questions of fertility and sterility of farm animals. Every one must admit the interest and importance of the subject, but no British physiologists except Mr Heape and Dr Marshall seem to have regarded it as a promising field for statistical and experimental enquiry.

The explanation probably lies in the lack of any book giving a systematic account of the work which has already been done and making the very scattered literature of the subject readily available to the would-be investigator.

Dr Marshall has supplied this want and deserves the gratitude of all interested in the breeding of animals.

His book contains much of interest to agriculturists of all shades, both practical and scientific, and should be especially useful to those who are concerned with reproduction from the investigator's point of view.

**The Feeding of Crops and Stock.** By A. D. HALL, M.A., F.R.S. [John Murray.] In this book, which is intended as an introduction to the more systematic study of what is commonly called Agricultural Chemistry, the author gives a clearly written account of the relations between plants and the air and soil, the building up process in the plant, and the utilization of crops for the feeding of animals. It is written in Mr Hall's easy and interesting style, and is good reading

alike for the serious student and the casual reader who is interested
in the problems of farming.   It may be especially commended to those
teachers who wish to give their students a sound introduction to the
general principles of plant and animal nutrition.   It is illustrated with
photographs and diagrams.   It is to be hoped that several mistakes
in the spelling of botanical names will be corrected in the next edition.

**A Text-Book of Egyptian Agriculture,** Vol. II.   Edited by
G. P. FOADEN, B.Sc. (Edin.), and F. FLETCHER, M.A. (Camb.), B.Sc.
(Lond.).   [National Printing Department, Cairo.]   The second volume
of this text-book of Egyptian Agriculture treats of Farm Seeds, Rota-
tions, Farm Crops, Horticulture, Fungoid and Insect Pests, Farm
Animals and Dairying.   The work which is by several writers is highly
instructive and in some cases very thorough.   Both the practical and
scientific aspects are treated of; and on the whole the former has been
written about with much judgement and common sense.   The article
on the insect enemies preying on the cotton crop shows much patient
research : the suggestions for their eradication are useful and the
author is, at any rate, not over sanguine as to the results of their
application.   It is somewhat to be regretted that in a work, in other
respects, so thorough, more space was not given to the treatment of
Farm Animals.

# THE EXPERIMENTAL ERROR OF FIELD TRIALS.

BY W. B. MERCER, B.Sc. (*Vans Dunlop Scholar, University of Edinburgh*), AND A. D. HALL, M.A., F.R.S.

*The Rothamsted Experimental Station.*

THE magnitude of experimental error attaching to one or more field plots is a question of extreme importance in Agricultural Science, because upon its proper recognition depends the degree of confidence which may be attached to the results obtained in field work. A very cursory examination of the results of any set of field trials will serve to show that a pair of plots similarly treated may be expected to yield considerably different results, even when the soil appears to be uniform and the conditions under which the experiment is conducted are carefully designed to reduce errors in weighing and measurement.

From the Rothamsted plots one of us has already shown (*Journ. Bd. Agric.* 1909, XVI, 365) that the probable error attaching to a single plot is in the neighbourhood of plus or minus 10 per cent. That is to say, that the chances are even against the result being within 10 per cent. of the truth, by the truth being meant the average result obtained by a number of experiments large enough to eliminate casual variations.

There are two methods of reducing the experimental error to a negligible amount, one by repeating the experiment over a long period of years, the method which has been adopted with the old experimental plots of Rothamsted, or secondly, by multiplying the number of plots on a given field. The first method does not eliminate causal variations induced by type of soil, the second is also subject to errors due to the season. For example, in manurial trials, phosphates are known to give higher returns in wet than in dry seasons, so that however accurately the value of particular phosphatic manurings might be determined by a series of plots in one year only, that value is only true for the season in question, and if a more general or average

value is to be obtained, the experiments must be prolonged until the variations due to season have been eliminated. Again, in a comparison of varieties certain varieties may be particularly suited to a heavy type of soil; hence a trial in a single field, however exactly it expressed the relative values of the two varieties on that land, cannot be taken as holding for more than the one type of soil. Of course the type of soil may be regarded as a factor that must always be expressed in connection with any statement as to the value of a given manure or a given variety, and it is fundamentally incorrect to try and smooth this effect out by averaging results obtained on different types of soils. For example, a given variety equally adaptable to all soils may have its value truly represented by the figure 100, and another variety may have a value of 130 on a heavy soil and only 70 on a light soil. To express these latter results by an average value of 100 is to introduce an unnecessary error, obscuring the real character of the variety. Seasonal variations, since they depend upon an unpredictable factor, cannot however be thus eliminated in making any statement about the value of a given manure or a variety. We are driven to content ourselves with a result that is true over an average of seasons, because a farmer is obliged to assume beforehand that he will obtain such an average season. Granting, however, the necessity of repeating an experiment for several years if any result that is finally true is to be obtained, there are yet many occasions and many types of experiments in which it is desirable to obtain reasonably accurate conclusions from a single season's work, and the practical point at issue is the size and number of plots which are most likely to reduce the inevitable error within working limits. In order to obtain light on this question, during the year 1910 an attempt was made at Rothamsted to estimate the variations in the yield of various sized plots of ordinary field crops which had been subjected to no special treatment and appeared to the eye sensibly uniform. The fields were selected, one of wheat which promised to be a fair crop for the season and was generally standing up well, the other of mangolds which looked a uniform and fairly heavy crop for the season and soil. In the wheat field a very uniform area was selected, one acre of which was harvested in separate plots, each one five-hundredth of an acre in area. The small sheaves which each plot yielded were then stored and eventually threshed out by a hand machine, corn and straw being separately weighed.

In measuring the plot a fixed number of rows of the drill were taken, eleven in this case, and a fixed length of 10·82 feet measured

TABLE I. *Plan and yields in lbs. of mangold plots; upper figures roots, lower leaves.*

**S.**

E. ← | → W.

| 376 | 371 | 355 | 356 | 335 | 332 | 335 | 340 | 352 | 352 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 67  | 52  | 50  | 50  | 48  | 44  | 51  | 50  | 46  | 49  |
| 316 | 338 | 336 | 356 | 332 | 340 | 339 | 359 | 365 | 349 |
| 56  | 51  | 48  | 50  | 49  | 48  | 47  | 48  | 52  | 46  |
| 326 | 326 | 335 | 343 | 330 | 328 | 350 | 339 | 337 | 362 |
| 56  | 47  | 47  | 49  | 47  | 47  | 52  | 47  | 50  | 46  |
| 317 | 343 | 330 | 327 | 336 | 340 | 335 | 301 | 348 | 357 |
| 54  | 54  | 50  | 46  | 45  | 51  | 47  | 44  | 46  | 52  |
| 321 | 332 | 317 | 318 | 306 | 331 | 335 | 311 | 329 | 353 |
| 50  | 46  | 44  | 47  | 48  | 46  | 47  | 47  | 46  | 49  |
| 335 | 349 | 332 | 358 | 325 | 348 | 328 | 290 | 326 | 323 |
| 56  | 51  | 44  | 48  | 45  | 49  | 48  | 41  | 44  | 44  |
| 341 | 346 | 321 | 321 | 314 | 340 | 337 | 321 | 329 | 317 |
| 57  | 52  | 46  | 48  | 45  | 50  | 52  | 45  | 44  | 41  |
| 347 | 363 | 349 | 311 | 329 | 321 | 337 | 300 | 364 | 310 |
| 59  | 58  | 49  | 51  | 46  | 46  | 45  | 46  | 50  | 45  |
| 354 | 365 | 350 | 339 | 316 | 342 | 335 | 331 | 345 | 327 |
| 64  | 54  | 49  | 47  | 42  | 49  | 49  | 48  | 51  | 47  |
| 309 | 352 | 328 | 297 | 308 | 313 | 349 | 372 | 362 | 346 |
| 55  | 56  | 49  | 43  | 40  | 46  | 54  | 51  | 55  | 51  |
| 349 | 335 | 323 | 325 | 322 | 348 | 350 | 351 | 350 | 360 |
| 60  | 50  | 48  | 43  | 46  | 54  | 50  | 51  | 52  | 51  |
| 343 | 346 | 300 | 312 | 313 | 331 | 347 | 324 | 336 | 339 |
| 57  | 52  | 41  | 48  | 42  | 51  | 47  | 46  | 46  | 44  |
| 338 | 327 | 323 | 296 | 311 | 335 | 320 | 318 | 343 | 333 |
| 53  | 49  | 45  | 38  | 40  | 47  | 44  | 44  | 44  | 45  |
| 339 | 384 | 322 | 331 | 277 | 299 | 332 | 302 | 318 | 306 |
| 52  | 61  | 46  | 44  | 37  | 43  | 50  | 39  | 42  | 44  |
| 314 | 325 | 330 | 328 | 301 | 316 | 338 | 310 | 304 | 302 |
| 52  | 46  | 48  | 44  | 40  | 43  | 46  | 45  | 42  | 37  |
| 300 | 329 | 322 | 310 | 309 | 342 | 350 | 335 | 320 | 278 |
| 52  | 50  | 48  | 45  | 40  | 49  | 51  | 46  | 42  | 38  |
| 369 | 341 | 344 | 324 | 316 | 342 | 351 | 324 | 310 | 309 |
| 60  | 51  | 47  | 44  | 44  | 51  | 53  | 46  | 47  | 44  |
| 298 | 302 | 325 | 300 | 286 | 330 | 324 | 322 | 309 | 322 |
| 49  | 47  | 47  | 46  | 40  | 52  | 49  | 43  | 45  | 49  |
| 324 | 316 | 310 | 317 | 295 | 308 | 295 | 267 | 307 | 290 |
| 52  | 48  | 45  | 44  | 40  | 45  | 54  | 40  | 43  | 41  |
| 330 | 306 | 306 | 306 | 291 | 321 | 325 | 288 | 302 | 310 |
| 57  | 48  | 45  | 41  | 41  | 45  | 48  | 41  | 46  | 42  |

**N.**

along the rows for cutting.   Thus, if there were any variation in the breadth of the drills the assumed one five-hundredth of an acre would not represent the actual land area of each plot.   These variations were small : moreover in all experimental crop work it is necessary to see that comparative plots do contain exactly the same number of drills, so as to make the measured area one of crop rather than of land.

For the second trial, mangolds, the area in the same way was divided up into plots one two-hundredth of an acre, leaves and roots being weighed separately.   As before, each unit plot contained the same number (three) of drills and was measured along the drill a fixed length of 30·25 feet.
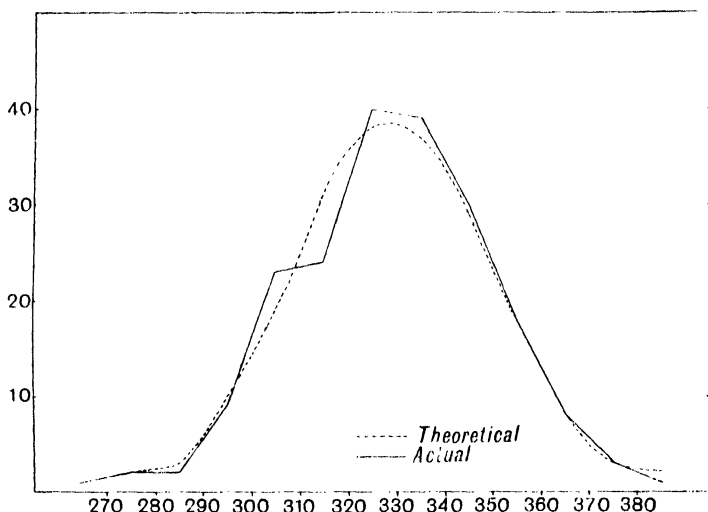


Fig. 1.   Frequency curve for 200 plots of mangold roots ; actual and theoretical.

It will be convenient to begin with mangold experiments which contain the smaller number of plots, but the principles discussed are the same in both cases :

*Table* I shows the weights of roots and leaves on all the plots as they stood in the field ; the length of the plots runs with the horizontal lines of figures, this being also the direction of the drills across the field. A first examination of the results shows that large variations occur in the weights of individual plots ; the highest yield of roots is 384 pounds and the lowest 267, variations of 18 per cent. on either side of the mean.   In order to get a general idea of the magnitude and nature of the variations it is convenient to plot them into a frequency curve, and

Fig. 1 shows the curve obtained by gathering the weights of the roots into groups at intervals of 10 lbs., together with the "normal curve of error," calculated to fit the observations. It will be seen that the two curves agree very closely, considering the small number of observations, and there is no sign that the material is not homogeneous. This enables us to apply the well-known properties of the normal curve of error to our results. The next thing which may be examined is whether the position of the plot in the field has any effect upon its yield, *i.e.* whether there is any uniform change in the character of the soil, etc., that brings about a steady change in the weights in passing either down or across the field. This can be roughly tested by adding together the weights on each plot as set out in the table, first horizontally and then vertically.

The results are set out in Table II, and expressed graphically by

TABLE II.   *Varying weight of rows of plots ; mangolds.*

| Total Weight of Rows in lbs. | |
| --- | --- |
| East to West | South to North |
| 6646 | 3504 |
| 6796 | 3430 |
| 6558 | 3376 |
| 6505 | 3334 |
| 6252 | 3253 |
| 6637 | 3314 |
| 6712 | 3287 |
| 6408 | 3361 |
| 6656 | 3404 |
| 6545 | 3366 |
| | 3416 |
| | 3291 |
| | 3244 |
| | 3210 |
| | 3168 |
| | 3195 |
| | 3330 |
| | 3118 |
| | 3029 |
| | 3085 |

the two curves in Fig. 2.   It is clear that there is a slight falling off in yield as one passes from South to North, but the variations from East to West are covered by the experimental error.   Where such a uniform change can be traced as shown from South to North in these experiments

it might be possible to apply a correction for position. But the device is cumbrous and the same end may be gained by duplication and reflection of the order of the plots about their centre. Thus duplicates of three plots would be arranged in a row in the order *a, b, c, c, b, a*. If the variation in the land is perfectly uniform the error due to position obviously disappears.

Further it must not be supposed that the results obtained for this set of plots in 1910 can be used to correct results obtained on the same plots in any other year. For example if any given area gives a yield of 10 per cent. over the mean in 1910 we cannot assume that we shall obtain a true average yield by deducting 10 per cent. from the yield of
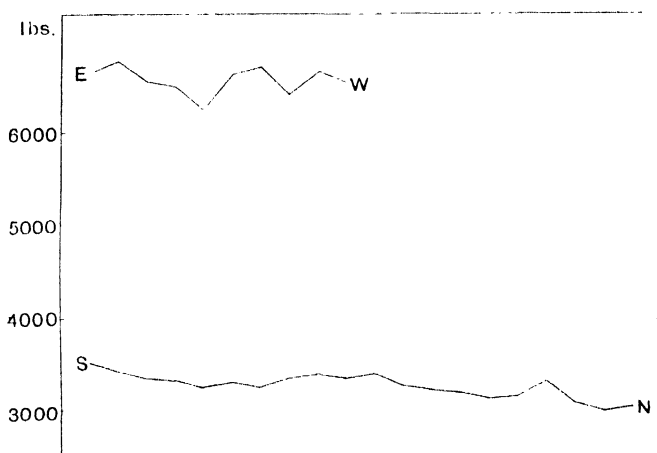


Fig. 2.   Varying weight of rows of plots with position in the field.   Mangolds.

the same plot in 1911. The variations, as we have learnt from other observations, are determined by so many incalculable factors that they may fairly be described as casual, and a plot above the average in one year is as likely as not to be below the average in the next year.

We may now test whether the weights of roots and leaves vary together. As the whole area had been similarly treated it might be expected that the variations in the weight of the leaves would follow fairly closely those of the roots. Moreover, if this fact can be established attention may be confined to the roots alone, as the same arguments will apply to both equally.

The plots were therefore gathered together into groups according to their weights, each group containing all the plots whose weights fell

within a range of 10 lbs., *e.g.* 379—370, 369—360, 359—350, etc.  The average weight of the roots of the plots in each of these groups was then found, this of course being somewhere about five in the unit, and also the average weight of the leaves that accompanied the roots on each of the plots in question.  Thus we obtain the average weight of leaves corresponding to the average weight of roots for a series of groups of plots giving regular increases in their yield of roots.  These results are set out in Table III and are plotted graphically in Fig. 3, forming

TABLE III.  *Relation between Roots and Leaves.*

| No. of Plots in Group | Mean Weight of Group in lbs. | | No. of Plots in Group | Mean Weight of Group in lbs. | |
|---|---|---|---|---|---|
| | Roots | Leaves | | Roots | Leaves |
| 1 | 267 | 40·0 | 39 | 334 | 48·3 |
| 2 | 277 | 37·5 | 30 | 344 | 50·8 |
| 2 | 287 | 40·5 | 18 | 353 | 51·1 |
| 9 | 295 | 43·3 | 8 | 364 | 53·3 |
| 23 | 304 | 44·1 | 3 | 373 | 56·6 |
| 24 | 314 | 45·5 | 1 | 384 | 61·0 |
| 40 | 324 | 46·6 | | | |

what is known as a Regression Line.  If the weight of leaves had been exactly proportional to the weight of roots we should obtain a straight line on the graph and it will be seen that the experimental material does yield approximately a straight line, the deviations being only considerable at the extremes of the graph, when the numbers of plots making up the group are smaller and the casual variations are not smoothed out.

The character of the graph shows us that the weights of leaves and roots vary together, so that further discussion may be confined to the roots.

The next step is to determine the standard deviation of the plots from the mean, the standard deviation being the square root of the sum of the squares of all the differences from the mean after dividing by one less than the total number of plots.  Two-thirds of the standard deviation gives us the probable error of a single plot, that is to say, the limits within which it is an even chance a particular plot will fall.  If *a*, *b*, *c*, etc., be the weights of each plot, *m* their mean, and *n* the number of plots, then

$$\text{S. D.} = \sqrt{\frac{(a-m)^2 + (b-m)^2 + (c-m)^2, \text{ etc.}}{n-1}}.$$

Probable error = S. D. × ·6745 or 2/3 S. D. approximately.

Working in this way the probable error attaching to a single plot of one two-hundredth of an acre in area is found to be 20·4 lbs., the mean being 328·6 lbs., or as previously explained, it is an even chance that any given plot weighs more than 308·2 and less than 349 lbs.
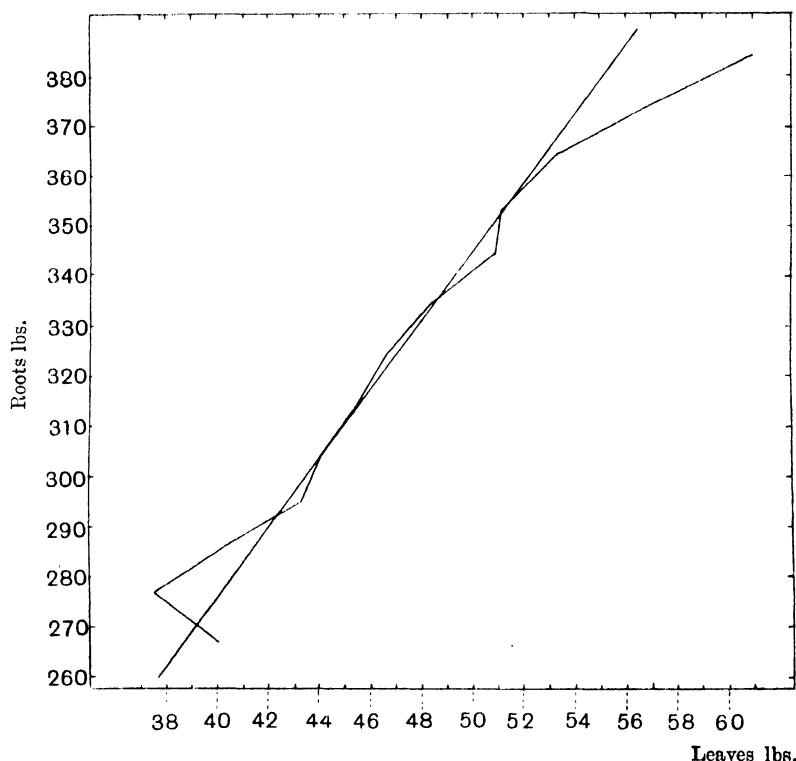


Fig. 3.   Regression line showing average weight of leaves for any weight of roots.

Calculating as a percentage of the mean the standard deviation becomes 6·2 per cent.   The next step is to group pairs of adjacent plots together to form single plots of twice the former length and one-hundredth of an acre in area.   When this is done the standard deviation is reduced to 5·36 per cent.   Again, grouping adjacent plots in pairs so as to obtain five plots in all from East to West, and 10 plots from North to South, thus making up areas of one-fiftieth of an acre, the standard deviation becomes 4·51 per cent. of the mean.   Grouping once more five adjacent plots, so as to obtain four larger plots from North to South and 10 from East to West, each one-fortieth of an acre

in area, the standard deviation becomes 4·06 per cent.; again grouping 10 adjacent plots together so as to get units of one-twentieth of the acre, the standard deviation falls to 3·6 per cent. of the mean, and is further reduced to 3 per cent. when the plots are made up to one-tenth of an acre[1]. It is clear that the probable error attaching to the result obtained for a single plot diminishes with the size of the plot. We can obtain some rather useful information by plotting the standard deviation in percentages against the size of the plot, which gives us
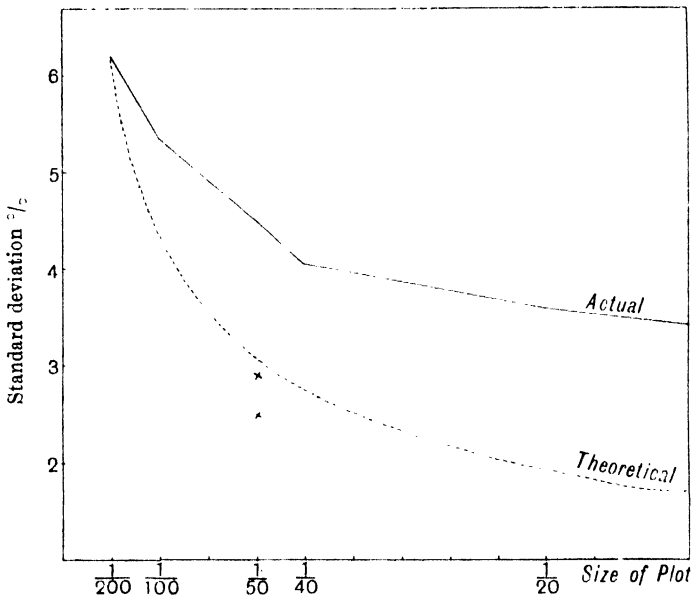


Fig. 4.   Percentage standard deviations with plots of different size ; mangolds.

the curve shown in **Fig. 4.** On the same figure is also drawn a theoretical curve that has been deduced on purely statistical grounds to give the standard deviation of successively larger plots made up of a number of the original one two-hundredths taken at random, *i.e.* not consecutively, nor in any other regular manner. The curve for the experimental results shows that the reduction of the probable error is not very rapid when a plot increases above a certain size. In fact the curve indicates that at about one-fortieth of an acre the

[1] As the number of plots decreases the value which can be attached to the determination of the S. D. diminishes, and in the later calculations when small numbers of plots are made up from scattered units, the results given are invariably averages of two or three determinations.

probable error attaching to a single plot has attained such a figure that it is not profitable to try and reduce it further by merely increasing the size of the plot.

As regards our standard plot then, one in the neighbourhood of one-fortieth of an acre is large enough.

We may next vary our groupings of the small one two-hundredth of an acre plots, so as to ascertain if the shape of the plot has any effect upon the result. This was done by making up plots of one-twentieth of an acre in two ways, one two plots long from East to West, and five plots deep from South to North (making a plot about 20 yards long along the drills and 12 yards broad), the other a long narrow plot, five of the small plots long from East to West, and two only deep from North to South, *i.e.* about 50 yards × 5 yards. For the first description of plot the standard deviation is 3·47 per cent., for the second 3·73 per cent., results from which little can be deduced as to any superiority of long and narrow plots over square ones.

Having thus ascertained the probable error attaching to plots of a particular size and shape it is now desirable to get some light upon the reduction in the probable error that can be effected by multiplying the number of plots. Multiplying the number of plots would be only equivalent to increasing their size if the ground were absolutely uniform, but as we have every reason to expect that the variations in the yield of individual plots are largely due to inequalities of the soil, these may be expected to be considerably smoothed out if the area making up the larger plot is sub-divided into a number of smaller plots scattered more or less regularly over the experimental area. The fact that the theoretical curve in Fig. 4 so quickly falls below the observed one, is evidence that some considerable correlation exists between the yield of adjacent plots.

We began by making up plots of one-fiftieth of an acre by grouping together the yields of four of the one two-hundredth of an acre plots scattered systematically. The first method of scattering adopted gave a standard deviation of 2·5 per cent. for the one-fiftieth of an acre plots; a second method of scattering in which the distribution was not quite so regular gave a standard deviation of 2·9 per cent. Comparing these with the previous results we see that the standard deviation attached to a plot of one-fiftieth of an acre has been reduced from 4·5 per cent. to 2·7 (mean) by taking a fiftieth of an acre in four scattered two-hundredths instead of in one block.

Thus a very fair measure of precision can be attained with as small

an area as one-fiftieth of an acre when it can be sub-divided into scattered plots. However, one two-hundredth of an acre is a very small plot for most kinds of field experiments, and we have already decided that the best size of plot comes somewhere in the neighbourhood of one-fortieth of an acre. Accordingly, a further calculation was made of the standard deviation of plots of one-tenth of an acre made up of four scattered fortieths. Two methods of scattering were adopted; one gave a standard deviation of 2·0 per cent., the other of 1·3 per cent.

Taking the mean of these figures [S. D. = 1·65 $^0/_0$], we find that tenth acre plots made up of four or five units scattered about the experimental field ought to yield us a result with a probable error of about one per cent. In other words, if the yield of this field is 30 tons per acre, then the weight obtained from five portions each one-fortieth of an acre taken at various places about the field, might be expected to fall between 30 tons 6 cwt. and 29 tons 14 cwt., the chances being even for and against such a measure of accuracy.

Again, as with simple plots the standard deviation for a plot made up of a number of smaller scattered units is not diminished in proportion to their increase in size, though for any given size of plot it is considerably reduced if the plot be made up of several scattered units. It is desirable to find whether the standard deviation will be decreased proportionately if the number of units into which an area is divided is increased. Determinations were therefore made of the standard deviation for plots of one-fifth of an acre made up of two-tenths, four-twentieths and ten-fiftieths respectively. The results are set out in Table IV, and expressed graphically in Fig. 5, standard deviation being plotted against number of units.

TABLE IV. *Standard deviation with different numbers of component units.*

| No. of units | Standard Deviation |
|---|---|
| 1 | 2·48 |
| 2 | 1·76 |
| 4 | 1·36 |
| 10 | 1·19 |

The figure shows that little is gained by increasing the number of scattered units beyond four or five, so that the results for one-tenth of an acre given above yield the lowest standard deviation which it is

economically possible to obtain. This is quite sufficient for ordinary experimental purposes, though, as we shall show later, in certain special cases where a very close comparison is necessary between a pair of varieties or two methods of treatment, a still greater precision can be obtained by a proper arrangement of the experimental areas.

It should be noted that the values obtained for the standard deviation for a given size and number of plots must not be interpreted too literally. They are only strictly applicable to the particular field, crop and season under discussion, because under other conditions the
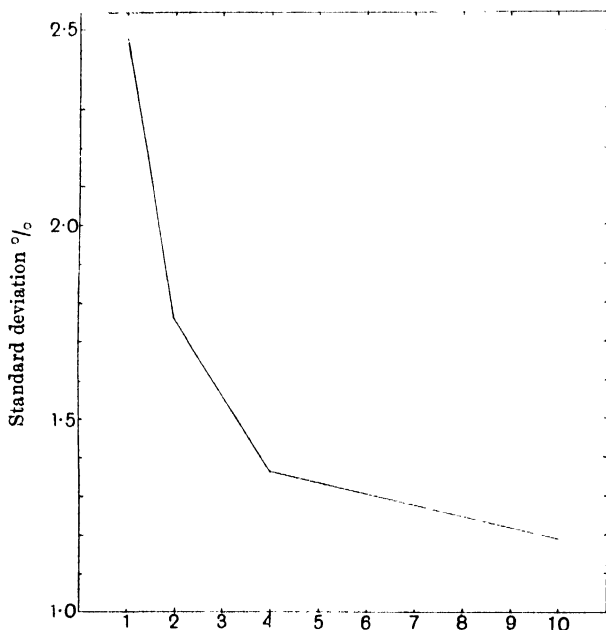


Fig. 5.   Variation of standard deviation with number of units in plot; mangolds.

factors inducing variation may become considerably altered. The principles, however, upon which they are founded hold in all cases, and the actual values obtained indicate in all probability the order of magnitude of the error that will be experienced.

We may now turn to the results obtained with wheat, where we have a larger number of plots, 500 in all. Table V shows the actual yields of grain and straw per plot in pounds, arranged as they occurred in the field. The maximum yield of grain is 5·16 lbs., the minimum 2·73 lbs., a variation of 30 per cent. on either side of the mean.

TABLE V. *Plan and weights in lbs. of 500 wheat plots; upper figures grain, lower straw.*

N.

(Each cell gives upper figure = grain, lower figure = straw. Orientation: N at top, S at right, E at left, W at bottom. Reading left to right, top to bottom.)

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4·53/6·78 | 4·23/6·08 | 2·97/4·53 | 3·17/4·95 | 3·36/4·76 | 3·72/5·47 | 4·27/6·54 | 3·51/5·05 | 3·57/5·12 | 3·66/5·96 | 3·64/5·11 | 3·92/6·33 | 4·58/7·23 | 4·02/6·10 | 4·37/6·25 | 4·00/5·87 | 4·14/6·98 | 4·04/6·08 | 4·75/7·31 | 4·48/6·08 | 3·04/4·71 | 5·13/7·99 | 4·06/7·19 | 4·15/6·85 | 3·63/6·37 |
| 3·94/5·68 | 4·09/5·92 | 3·14/5·11 | 3·53/5·09 | 3·69/5·56 | 4·14/6·14 | 4·26/8·61 | 3·53/5·83 | 3·44/5·00 | 3·82/5·80 | 3·61/5·58 | 3·97/6·03 | 4·05/6·57 | 4·19/6·43 | 3·97/6·09 | 4·50/6·50 | 4·08/6·94 | 4·08/6·35 | 4·56/7·88 | 3·74/6·63 | 4·03/6·34 | 4·64/7·80 | 4·15/7·41 | 4·21/7·29 | 4·07/6·24 |
| 4·88/7·49 | 3·97/6·28 | 3·59/5·41 | 3·66/6·15 | 4·33/6·17 | 4·31/6·14 | 4·20/6·55 | 3·53/4·91 | 3·44/5·56 | 4·07/6·74 | 3·64/5·86 | 4·58/6·62 | 3·73/6·02 | 4·07/6·37 | 3·94/6·18 | 3·67/6·20 | 3·30/5·95 | 3·76/5·93 | 4·76/8·18 | 4·46/6·98 | 3·77/6·17 | 4·69/7·50 | 4·40/7·35 | 4·29/7·71 | 4·51/7·05 |
| 3·64/6·55 | 3·46/5·41 | 3·32/4·62 | 3·89/5·92 | 3·81/6·13 | 4·10/5·96 | 4·30/5·95 | 3·77/5·79 | 3·97/6·03 | 4·54/7·08 | 3·75/4·62 | 3·19/6·56 | 4·06/6·19 | 3·34/5·60 | 4·01/5·99 | 4·59/5·41 | 3·05/5·82 | 4·52/7·29 | 4·52/7·60 | 3·91/6·46 | 3·49/5·70 | 4·04/6·66 | 4·05/7·89 | 4·64/8·23 | 3·90/6·91 |
| 4·81/6·56 | 3·96/5·60 | 3·57/5·24 | 3·67/5·45 | 3·55/5·89 | 3·74/5·76 | 4·29/6·15 | 4·29/5·58 | 3·79/5·33 | 4·14/6·14 | 3·70/7·67 | 4·14/5·98 | 3·74/6·13 | 3·63/6·43 | 3·92/6·75 | 5·07/8·05 | 3·67/7·33 | 4·17/7·33 | 4·40/7·91 | 4·10/6·77 | 3·61/6·05 | 4·64/7·86 | 4·92/8·58 | 4·27/7·73 | 3·63/5·93 |
| 4·29/6·15 | 2·78/4·28 | 4·24/5·82 | 3·94/5·87 | 4·20/5·92 | 3·58/5·89 | 4·24/5·88 | 4·35/6·09 | 3·87/5·57 | 4·01/5·99 | 3·37/5·00 | 3·76/5·49 | 3·72/6·03 | 4·09/6·10 | 3·79/5·46 | 4·36/5·55 | 4·26/6·61 | 3·84/6·28 | 4·89/6·86 | 3·66/6·06 | 3·61/5·89 | 4·73/6·98 | 4·08/7·04 | 3·55/6·45 | 3·16/5·59 |
| 3·44/5·68 | 3·47/5·59 | 3·44/4·56 | 3·05/4·57 | 3·42/5·45 | 4·06/6·25 | 3·81/5·69 | 4·38/6·24 | 3·97/6·03 | 4·59/6·49 | 3·71/5·66 | 3·77/5·48 | 4·05/6·82 | 3·74/6·38 | 3·96/6·23 | 4·86/7·51 | 4·32/7·37 | 4·06/6·81 | 4·91/8·06 | 3·56/6·06 | 3·28/4·97 | 4·39/6·98 | 4·28/7·02 | 3·50/5·87 | 3·18/5·32 |
| 4·24/7·26 | 3·47/5·59 | 4·17/5·58 | 3·63/5·81 | 3·95/5·92 | 4·41/6·15 | 3·85/5·21 | 3·71/5·91 | 3·75/5·12 | 4·76/6·49 | 3·71/5·79 | 3·93/6·07 | 3·72/5·97 | 3·91/6·36 | 3·93/6·13 | 4·77/7·57 | 4·13/7·24 | 3·92/6·70 | 4·02/8·02 | 3·54/6·46 | 3·19/5·33 | 4·66/7·28 | 4·41/7·05 | 3·35/5·71 | 3·97/6·03 |
| 4·55/6·32 | 3·44/4·81 | 3·97/5·65 | 4·03/5·72 | 4·21/6·04 | 4·41/6·15 | 4·09/5·47 | 4·21/6·29 | 4·24/6·45 | 4·73/6·64 | 3·75/5·12 | 3·96/6·35 | 4·05/6·82 | 3·59/6·03 | 3·56/5·75 | 5·02/8·23 | 4·37/7·37 | 2·99/5·07 | 4·64/7·92 | 4·11/6·88 | 3·75/5·94 | 4·49/6·95 | 4·67/7·17 | 3·61/6·01 | 3·40/5·66 |
| 3·91/5·96 | 2·84/4·10 | 3·89/5·98 | 4·03/5·72 | 4·08/7·00 | 4·20/6·49 | 4·09/6·16 | 4·07/6·18 | 4·09/6·16 | 4·20/6·49 | 3·09/5·47 | 3·47/5·78 | 3·91/6·25 | 3·99/6·36 | 3·86/6·14 | 4·44/7·25 | 3·99/7·26 | 3·61/6·33 | 4·66/7·59 | 4·20/6·86 | 4·13/7·31 | 4·42/6·95 | 4·27/7·17 | 3·71/6·29 | 3·39/5·61 |
| 3·29/5·65 | 3·47/5·84 | 3·89/5·98 | 4·08/6·54 | 4·11/6·58 | 3·73/6·89 | 4·03/7·16 | 3·82/7·30 | 3·57/6·55 | 3·39/6·42 | 3·73/6·58 | 3·29/5·71 | 4·09/7·28 | 3·82/7·30 | 4·17/7·52 | 4·56/7·73 | 3·72/7·28 | 4·08/7·17 | 4·45/8·74 | 4·37/8·75 | 3·59/6·53 | 4·45/7·17 | 3·82/6·87 | 3·70/6·17 | 4·43/7·07 |
| 3·88/6·29 | 3·56/6·38 | 3·80/5·70 | 3·69/5·70 | 3·22/5·40 | 3·86/5·80 | 3·86/5·64 | 3·47/5·85 | 2·92/4·95 | 3·60/5·80 | 3·30/5·70 | 3·05/5·70 | 3·91/5·82 | 3·55/5·70 | 4·99/7·82 | 4·77/7·65 | 3·86/7·20 | 3·92/6·70 | 4·42/8·02 | 3·54/6·46 | 3·94/7·06 | 4·57/7·93 | 4·41/6·99 | 3·79/6·33 | 4·52/7·10 |
| 4·33/7·11 | 3·93/5·69 | 4·02/6·35 | 4·58/6·79 | 3·93/6·57 | 4·54/6·64 | 3·67/6·20 | 3·09/5·33 | 4·13/6·87 | 3·60/6·27 | 3·14/5·48 | 3·05/5·70 | 3·91/6·21 | 3·55/6·70 | 4·77/7·84 | 4·63/7·87 | 3·82/7·05 | 3·44/6·70 | 4·37/8·02 | 4·47/8·53 | 4·29/7·08 | 4·49/7·93 | 4·67/7·03 | 4·09/7·22 | 4·46/7·16 |
| 3·93/6·07 | 3·47/5·78 | 4·39/6·11 | 4·08/6·54 | 4·24/7·20 | 4·20/6·49 | 4·36/7·26 | 3·48/5·52 | 3·19/5·87 | 3·39/6·42 | 3·73/6·58 | 3·29/5·71 | 3·60/7·00 | 3·82/7·30 | 4·17/7·52 | 4·63/7·57 | 3·73/6·89 | 4·11/7·58 | 4·37/8·15 | 3·96/7·10 | 4·31/7·08 | 4·31/7·31 | 4·39/7·73 | 4·42/7·22 | 3·46/8·85 |
| 3·38/5·68 | 3·43/5·82 | 3·69/5·18 | 3·25/6·37 | 3·71/6·29 | 3·65/6·60 | 3·05/5·20 | 3·48/5·52 | 3·08/5·42 | 3·39/6·42 | 3·14/5·68 | 3·09/5·33 | 3·57/6·55 | 3·43/5·38 | 4·09/7·03 | 4·03/7·16 | 3·73/6·89 | 4·11/7·58 | 3·89/6·86 | 3·85/7·10 | 4·01/7·18 | 4·08/6·67 | 3·84/6·60 | 5·16/8·78 | 5·13/8·37 |
| 3·63/5·99 | 3·79/5·43 | 3·80/5·70 | 3·69/5·93 | 3·22/5·40 | 3·86/5·64 | 3·86/5·80 | 3·09/5·41 | 2·85/4·96 | 3·60/5·80 | 3·14/5·68 | 3·60/5·99 | 3·75/6·31 | 3·55/6·70 | 4·06/6·34 | 4·61/7·51 | 4·21/7·24 | 4·41/7·32 | 3·91/6·91 | 3·94/6·73 | 4·84/8·85 | 3·92/6·70 | 4·23/7·14 | 5·09/8·72 | 4·23/6·59 |
| 4·06/6·88 | 3·84/6·35 | 3·69/5·43 | 3·91/6·09 | 3·69/6·57 | 3·22/5·40 | 3·66/5·84 | 3·36/6·14 | 3·49/5·82 | 3·14/5·68 | 3·01/5·68 | 3·24/5·95 | 3·54/5·58 | 3·70/6·80 | 4·44/6·87 | 4·46/6·91 | 4·15/7·47 | 4·24/7·32 | 4·41/7·51 | 4·38/7·43 | 4·46/7·54 | 3·66/7·09 | 4·23/7·14 | 5·09/8·72 | 4·38/6·72 |
| 3·67/6·33 | 3·79/6·01 | 3·69/5·43 | 3·76/6·11 | 3·91/7·50 | 3·48/6·13 | 3·66/5·84 | 3·09/5·41 | 2·73/4·77 | 2·85/4·96 | 3·60/5·99 | 3·01/5·65 | 3·60/5·58 | 3·14/5·48 | 4·06/6·91 | 1·91/6·91 | 4·15/7·47 | 1·37/7·32 | 4·41/7·51 | 4·38/7·43 | 4·06/7·06 | 3·66/7·09 | 4·42/8·45 | 3·85/8·78 | 3·85/6·59 |
| 4·19/6·93 | 3·76/6·36 | 3·59/5·21 | 3·54/6·21 | 3·71/6·35 | 3·60/6·21 | 3·41/6·28 | 3·49/5·82 | 3·29/5·95 | 4·19/7·30 | 5·13/8·31 | 3·24/5·95 | 3·70/6·80 | 4·28/6·97 | 4·49/7·57 | 4·46/6·91 | 4·21/7·41 | 4·15/7·47 | 4·52/7·73 | 4·38/7·43 | 4·84/8·85 | 5·16/8·78 | 4·69/8·06 | 4·28/7·03 | 3·61/6·20 |
| 3·36/6·33 | 3·76/6·36 | 3·59/4·66 | 3·54/6·21 | 3·71/6·35 | 3·24/5·58 | 3·29/5·95 | 3·70/6·80 | 4·52/8·17 | 4·44/6·87 | 3·01/5·68 | 3·24/5·95 | 3·49/5·82 | 4·28/6·97 | 4·44/6·87 | 4·46/6·91 | 3·89/7·36 | 3·97/7·53 | 4·06/7·06 | 4·22/7·72 | 3·66/7·09 | 5·09/8·72 | 4·42/8·45 | 4·22/7·65 | 3·61/6·20 |

S.

Fig. 6.   Regression line showing average weight of straw at any weight of grain.
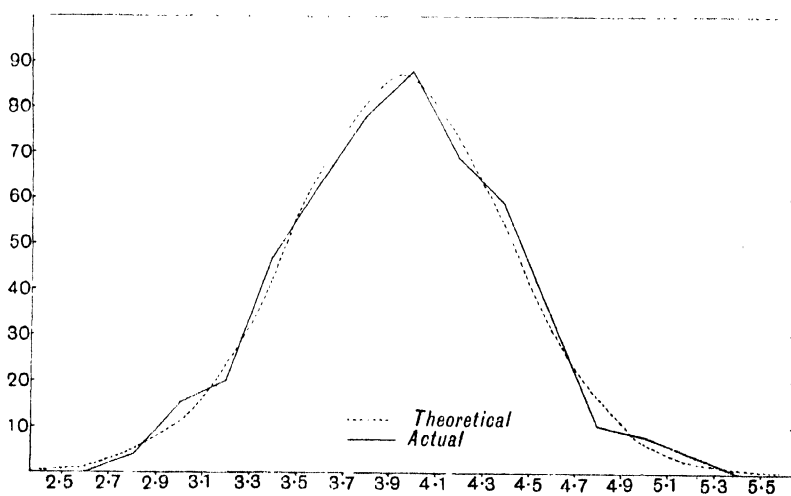


Fig. 7.   Frequency curve; wheat.

Table VI and Fig. 6 show the relation of grain to straw, obtained by the method previously described, the plots having been grouped according to the weights of grain, at intervals of 0·1 lbs., *e.g.* 2·7—2·79, 2·8—2·89, 2·9—2·99, etc., and the average weights of grain and straw on the plots falling in each group being determined. Again a somewhat irregular regression line is obtained, though it lies sufficiently close to the straight line to show that the yields of grain and straw do vary together, and that there is no tendency to such an inverse variation as would cause the straw to be light where the grain is heavy. Hence it will be sufficient to deal with the grain alone.

TABLE VI. *Relation between grain and straw.*

| No. of Plots in Group | Mean Weight of Group in lbs. | | No. of Plots in Group | Mean Weight of Group in lbs. | |
|---|---|---|---|---|---|
| | Grain | Straw | | Grain | Straw |
| 2 | 2·75 | 4·53 | 42 | 4·06 | 6·61 |
| 2 | 2·85 | 4·53 | 28 | 4·14 | 6·90 |
| 3 | 2·96 | 4·85 | 41 | 4·24 | 6·90 |
| 12 | 3·08 | 5·55 | 31 | 4·36 | 6·95 |
| 10 | 3·17 | 5·56 | 28 | 4·44 | 7·36 |
| 10 | 3·26 | 5·59 | 22 | 4·55 | 7·25 |
| 18 | 3·36 | 5·73 | 13 | 4·66 | 7·76 |
| 29 | 3·45 | 5·70 | 7 | 4·76 | 7·79 |
| 26 | 3·55 | 5·83 | 3 | 4·85 | 7·58 |
| 37 | 3·65 | 5·92 | 5 | 4·91 | 8·00 |
| 44 | 3·74 | 6·17 | 3 | 5·06 | 8·33 |
| 34 | 3·85 | 6·40 | 4 | 5·14 | 8·36 |
| 46 | 3·94 | 6·42 | | | |

Following the previous method of treatment we may now begin by plotting the result into the frequency curve, Fig. 7. This curve fits the theoretical one as well as may be expected considering the number of observations, so we may conclude that the material is fairly homogeneous. Adding again the weights from the plots in rows from East to West, and from North to South, the results of which are expressed in Table VII and Fig. 8, we again see evidence of casual irregularity. There is evidently no systematic variation of soil from East to West, and though from North to South there seems to be a decline in yield it is too irregular to be made a basis of any corrections. Certain systematic correlations were also tested. The field was laid up in lands 15—17 yards broad. There were four (or sometimes only

TABLE VII.    *Varying weight of rows, wheat.*

| Total Weight of Rows in lbs. | |
| --- | --- |
| North to South | West to East |
| 98·9 | 78·3 |
| 100·2 | 79·3 |
| 101·1 | 85·6 |
| 97·9 | 90·8 |
| 100·7 | 76·4 |
| 98·8 | 81·1 |
| 96·8 | 89·5 |
| 97·0 | 79·3 |
| 102·0 | 78·8 |
| 97·8 | 90·6 |
| 98·9 | 82·3 |
| 99·6 | 77·9 |
| 102·9 | 77·9 |
| 98·4 | 74·8 |
| 92·7 | 72·2 |
| 93·6 | 77·3 |
| 97·5 | 70·6 |
| 97·9 | 75·1 |
| 104·7 | 77·5 |
| 96·5 | 80·1 |
| | 76·3 |
| | 75·2 |
| | 75·9 |
| | 73·1 |
| | 78·9 |
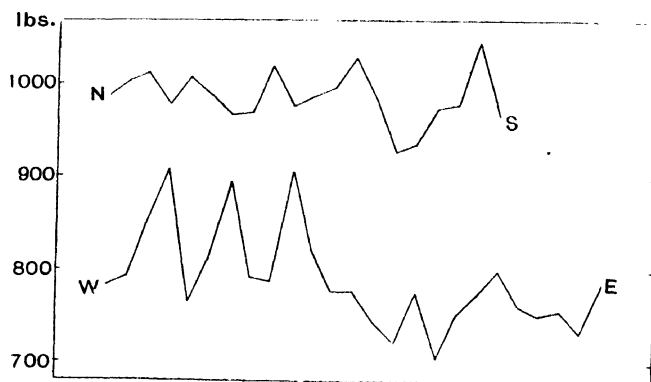


Fig. 8.    Varying weight of rows of plots with position in field ; wheat.

three) rows of plots on each land, a strip of varying breadth containing the actual furrow being left uncut between each row of plots. It was thus possible to obtain a comparison between the plots occupying the crown of the ridge and the neighbouring plots adjoining though not actually containing a furrow. Table VIII shows the mean result obtained in each case, from which it will be clear that the situation of the plots on the crown of the ridge or near the furrow had no effect upon their yield. This result is somewhat surprising, because to the eye the plots upon the ridge appeared to be decidedly better, an illusion we may assume to have been caused by the wheat ears on the ridge being lifted up to the eye.

TABLE VIII. *Influence of position on land.*

| No. of Land | Mean Weight of Plots in lbs. | |
| --- | --- | --- |
| | Middle of Land | Sides of Land |
| 1 | 4·12 | 4·22 |
| 2 | 4·05 | 4·15 |
| 3 | 3·94 | 4·24 |
| 4 | 3·89 | 3·92 |
| 5 | 3·70 | 3·68 |
| 6 | 3·91 | 3·82 |
| 7 | 3·80 | 3·79 |
| Mean | 3·91 | 3·97 |

Again, one part of the area was a good deal contaminated with thistles, and notes were made at the time of cutting of the abundance of thistles or otherwise, in each plot. Altogether there were 187 plots in which the thistles were distinctly prominent, and these gave an average yield of 3·79 lbs. of grain, as against 4·04 lbs. of grain on 313 plots containing few or no thistles. The standard deviation of the difference may be calculated from the S. D. of the whole series of plots (0·46 lbs.) as follows: $0·46 \sqrt{\frac{1}{187} + \frac{1}{313}}$ or ·041 lbs., so that the probable error of the difference is ·027 lbs. Thus the presence of thistles had depressed the yield by $0·25 \pm 0·027$ lbs., or 6·16 per cent., a result which we can accept with some confidence because it is 9 times greater than the probable error. This appears to be a very small reduction of yield for a very marked contamination of the plots

with thistles, but it confirms the opinion of many farmers that thistles do less injury to a corn crop than other weeds like couch.

Every operation in harvesting must result in additional error, and since grain and straw vary together, it seemed possible that the weights of total produce would give a better idea of the relative value of different plots, or that the standard deviation calculated for grain + straw would be smaller than that for grain alone. But the standard
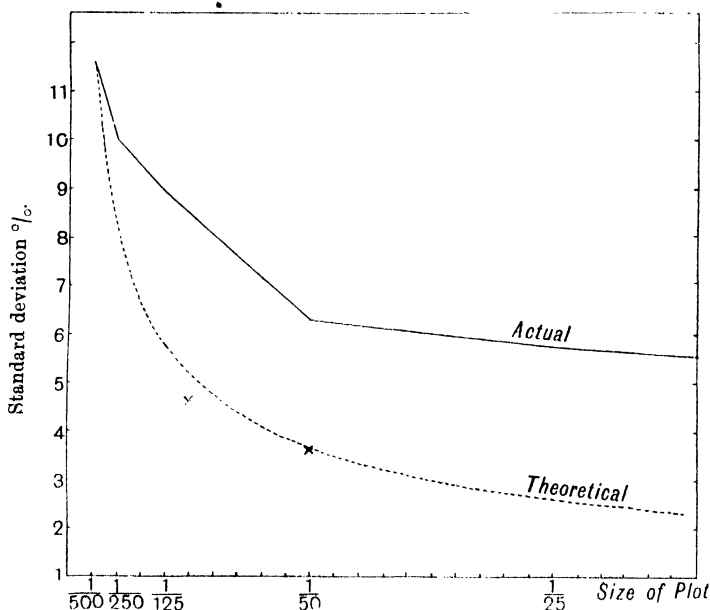


Fig. 9.   Standard deviation in plots of different sizes; wheat.

deviation of the total produce proved to be slightly higher, viz. 11·9 per cent., this being intermediate between 11·6 per cent. for grain and 13·8 per cent. for straw.

We may next proceed to calculate the standard deviation and probable error for plots of various sizes, using, however, this time an abbreviated method for obtaining the standard deviation which is described in a footnote[1]. We thus obtain a standard deviation of

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[1] Group the plots as in construction of a frequency curve. Select the group in which the mean is expected to lie (any other group may be taken without altering the result but the arithmetic is generally less arduous if the origin is taken near the mean) and from this as origin index the groups positively and negatively. Multiply the number in each group by the index number of the group. Sum and divide by the total number of observations. This gives $v_1$. Next multiply the number in each group by the square of the

11·6 per cent. for the smallest plot of one five-hundredth of an acre, 10 per cent. for one two-hundred-and-fiftieth of an acre, 8·9 per cent. for one-hundred-and-twenty-fifth, 6·3 per cent. for one-fiftieth of an acre, 5·7 per cent. for one-twenty-fifth, and 5·1 per cent. for one-tenth of an acre. These figures are gathered together in Fig. 9 and are compared with a theoretical curve calculated from the standard deviation of one five-hundredth acre, on the assumption that the material is homogeneous and subject to no systematic error. It will be seen that the standard deviations are larger for wheat than for mangolds when plots of the same size are compared, a difference which may be due to the crop and the nature of the soil in the two cases.

The graph also shows a very sharp change of curvature at about one-fiftieth of an acre, from which we may conclude that there is little

index number. Sum, and divide by the total number of observations. This gives $\nu_2$. Then S. D. $= \sqrt{\nu_2 - \nu_1{}^2 - \tfrac{1}{12}} \times$ unit of grouping.

In order to compare standard deviations derived from samples of varying size this value may be multiplied by $\sqrt{\dfrac{n}{n-1}}$, but as soon as this begins to make any appreciable difference it is a sign that the sample is growing too small to give an accurate value of the standard deviation.

$\nu_1 \times$ unit of grouping represents the difference between the true mean and the mid-point of the selected group. In case there are only a few observations the group unit should be small or there may be an appreciable difference between the mean and the S. D. as determined from the grouped figures and the actual figures. Where the number is large the error introduced in this way is negligible. For example the mean and S. D. found below from groups of 0·10 are 3·945 and 0·458, while from the same observations from groups of 0·20 they are 3·944 and 0·460.

*E.g.* 500 wheat plots

| | | | |
|---|---|---|---|
| 3·50 - 3·59 | 26 × − 4 = − 104 × − 4 = 416 |
| 3·60 − 3·69 | 37 × − 3 = − 111 × − 3 = 333 |
| 3·70 − 3·79 | 44 × − 2 = − 88 × − 2 = 176 |
| 3·80 − 3·89 | 34 × − 1 = − 34 × − 1 = 34 |
| 3·90 − 3·99 | 46 × 0 |
| 4·00 − 4·09 | 42 × 1 = 42 × 1 = 42 |
| 4·10 − 4·19 | 28 × 2 = 56 × 2 = 112 |
| 4·20 − 4·29 | 41 × 3 = 123 × 3 = 369 |
| 4·30 − 4·39 | 31 × 4 = 124 × 4 = 496 |
| | Total − 2 10542 |

$$\text{S. D.} = \sqrt{\frac{10542}{500} - \left(\frac{-2}{500}\right)^2 - \frac{1}{12}} \times \cdot 1 \times \sqrt{\frac{500}{499}} = \cdot 458.$$

$$\text{Mean} = 3\cdot945 - \tfrac{2}{500} \times \cdot 1 = 3\cdot945.$$

We have not grouped the observations in any case where the number of plots was less than 100.

advantage to be obtained by increasing the size of plots of wheat above one-fiftieth of an acre.  We may again try the effect of scattering the plots.  Making up plots of one-hundredth of an acre from five scattered plots each one five-hundredth of an acre, we obtain a standard deviation of 4·6 per cent.  Doubling the size of the plots and making up one-fiftieth of an acre in five scattered plots, each one-two-hundred-and-fiftieth of an acre, the standard deviation becomes 3·6 per cent. This is reduced to 2·4 per cent. when the area is made up to one-tenth of an acre in five scattered fiftieths, and it is only reduced to 1·97 per cent. when the plot is made one-fifth of an acre in five scattered twenty-fifths.

To test the number of scattered units into which it is most economical to divide a given area, the standard deviation of one-fifth acre plots made up in various ways was calculated.  The results are set

TABLE IX.  *Standard deviation in one-fifth acre plots composed of different numbers of units; wheat.*

| No. of units | Standard Deviation per cent. |
|---|---|
| 1 | 4·60 |
| 2 | 2·55 |
| 5 | 1·97 |
| 10 | 1·57 |

out in Table IX, and confirm the fact that it is undesirable to increase the number of duplicates beyond four or five.

Thus we confirm the evidence afforded by the standard deviation graph that the probable error is reduced sufficiently near to the minimum when the plots are one-fiftieth of an acre in area, beyond which point increase of size does not secure much greater accuracy. From five scattered plots each one-fiftieth of an acre in area we may expect to get a result with a probable error of about 1·6 per cent., or with a 40 bushel yield the odds are even that the weight from such an experimental plot will lie within 39·4 and 40·6 bushels.

It will be seen that these results for wheat agree with those previously obtained for mangolds, that the error attached to a single plot cannot be greatly reduced by increasing its area above one-fortieth or one-fiftieth of an acre, and that the probable error can be reduced to a working minimum by taking four or five similar plots scattered about

the field under experiment. We are indebted to "Student," by whose assistance and criticism we have been greatly aided in the whole of this discussion of our experimental results, for the working out of a method whereby the experimental error may be still further reduced when only a single comparison is desired, as for example between two varieties or two methods of manuring, by taking advantage of the correlation which exists between adjacent areas. This contribution is set out in an Appendix. Meantime the following general conclusions may be drawn from our results :

(1)   In all field trials, however uniform the land and careful the management of the experiment, there will be an experimental error attached to the result, due to so many incalculable factors that it may be described as casual.

(2)   The error diminishes with the size of the plot, but the reduction is small when the plot grows above one-fortieth of an acre.

(3)   The error may be best diminished by increasing the number of plots similarly treated and scattering them about the area under experiment, but there is not much to be gained by increasing the number of plots above five.

(4)   For practical purposes the authors recommend that in any field experiment each unit of comparison (variety, method of manuring, etc., according to the subject of the experiment) should be given five plots of one-fortieth of an acre each, systematically distributed within the experimental area.

This will reduce the experimental error to within two per cent. of the result, if the land is at all suited for experiment ; it does not however eliminate variations due to the unequal effects of different seasons upon the varieties or the action of the manures under experiment. Such variations can only be eliminated by continuing the experiments for several years. Similarly variations induced by the type of soil can only be ascertained by repeating the experiments on several soils.

# APPENDIX.

Note on a method of arranging plots so as to utilise a given area of land to the best advantage in testing two varieties.

THE authors have shown that to reduce the error as low as possible it is necessary to "scatter" the plots. I propose to deal with this point in the special case when a comparison is to be made between only two kinds of plots, let us say two varieties of the same kind of cereal.

If we consider the causes of variation in the yield of a crop it seems that broadly speaking they are divisible into two kinds.

The first are random, occurring at haphazard all over the field. Such would be attacks by birds, the incidence of weeds or the presence of lumps of manure. The second occur with more regularity, increasing from point to point or having centres from which they spread outwards; we may take as instances of this kind changes of soil, moist patches over springs or the presence of rabbit holes along a hedge.

Having made this distinction between random and regular causes of variation let me hasten to add that almost all causes of variation may belong to one or other or both of these classes according to the size of the plot in question.

In any case a consideration of what has been said above will show that any "regular" cause of variation will tend to affect the yield of adjacent plots in a similar manner; if the yield of one plot is reduced by rabbits from a bury near by, the plot next it will hardly escape without injury, while one some distance away may be quite untouched and so forth. And the smaller the plots the more are causes of variation "regular"; for example, with large plots a thistly patch may easily occur wholly within a single plot leaving adjacent plots nearly or altogether clean, but with quite small plots one which is overgrown with thistles is almost sure to have neighbours also affected.

Now if we are comparing two varieties it is clearly of advantage to arrange the plots in such a way that the yields of both varieties shall be affected as far as possible by the same causes to as nearly as possible an equal extent.

To do this it is necessary, from what has been said above, to

compare together plots which lie side by side and also to make the plots as small as may be practicable and convenient.

There is a reason, apart from the difficulty of cultivating very small plots, why the plots should not be made too small and that is, that when two different varieties are sown next one another the outside drill of each is under abnormal conditions and if it be counted in the plot may introduce an error which in a small plot may be quite substantial, but if it is not counted the space wasted by rejecting the outside drills of small plots becomes considerable.

Let us suppose that the smallest practicable size of plot has been chosen and the land available for the comparison has been divided up into plots of this size and sown, chequer fashion, with seed of the two varieties.

Obviously nothing that we can do (supposing of course careful harvesting) can now alter the accuracy of the resulting comparison of yields, but we can easily make different estimates of the reliance which we can place on the figures.

For example, the simplest way of treating the figures would be to take the yields of the plots of each variety and determine the standard deviation of each kind. Then from published tables we can judge whether such a difference as we find between the total yields is likely to have arisen by chance.

An advance on this is to compare each plot with its neighbour and to determine the standard deviation of the differences between these pairs of adjacent plots.

From what has been said above as to the occurrence of "regular" sources of error it will be seen that such differences as these will be to a much larger extent dependent on the variety, and to a less extent on errors, than if the mere aggregates are compared.

The standard deviation will therefore be smaller and the confidence which can be placed in the result increased.

By a further device we can still further decrease the standard deviation and increase our certainty.

For if, instead of harvesting the whole of each plot together, we divide each plot into two before harvesting (and that this can be done is clear from the account of the work done with the mangolds and wheat), then we get twice the number of comparisons, and the plots being half the size are comparatively closer together and the error of their comparison is reduced.

But, it will be asked, why take all this trouble? The error of

comparing plots of any given size has been found by the authors of the paper, and all that has to be done is to apply this knowledge to the particular set of experiments.

The answer to this is that there is no such thing as the absolute error of a given size of plot. We may find out the order of it, be sure perhaps that it is not likely to be less than (say) five per cent. nor more than 15 per cent. without producing visible heterogeneity, but the error of a given size of plot must vary with all the external conditions as well as with the particular crops upon which the experiment is being conducted, and it is far better to determine the error from the figures of the experiment itself; only so can proper confidence be placed in the result of the experiment.

The diagram illustrates the proposed method of arranging the plots.
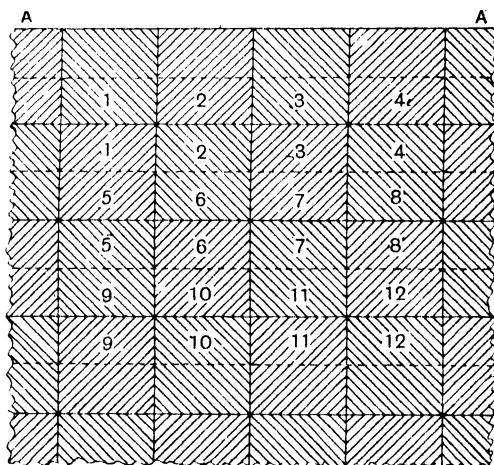


The different shading represents the two different varieties.

The firm lines represent the outside of the original plots.

*A A'* is part of the boundary of the experimental ground, part of which is given in the diagram.

The dotted lines show the further division made at harvesting.

Then the yields of the half-plots 1, 1 : 2, 2 : ... etc., are compared together.

The outside half-plots are neglected as it is usual to discard the edge of the field.

I have determined the error of comparing plots of different sizes in this way both with the mangold and the wheat figures.

Considering first the mangolds:

The crop on half an acre in the present experiment was about 32,860 lbs., and the standard deviation of a single one-two-hundredth acre was found to be 20·37 lbs. Hence the standard deviation of half an acre made up at random from 100 such small plots would be 20·37 × √100 or 203·7 lbs., and the standard deviation of the comparison between two such half-acres would be 203·7 × √2 or 287 lbs.

This would amount to ·87 per cent. so that one could not begin to be sure that a difference between two varieties of mangolds compared in this way (one-two-hundredth plots arranged at random) until it amounted to say 2·6 per cent.

But now suppose that the plots were each originally one-hundredth acre, bisected at harvest and compared as suggested above.

Then the actual figures given by the authors enable us to determine the standard deviation of the difference between the half-acre.

It amounts to no more than 223 lbs. or ·68 per cent. I.e. although working with plots twice the size up to harvest time we get the same accuracy with one acre of ground as would have been obtained with $\left(\frac{·86}{·68}\right)^2$ acres or 1·65 acres on the first plan.

Now suppose the plots to be one-fiftieth divided into one-hundredths at harvest.

Then I find the S. D. to be 274 lbs. or ·83 per cent.

Similarly $\frac{1}{75}$th acre plots harvested as $\frac{1}{150}$ths give a S. D. of comparison 289 lbs. or 0·88 %

| | | | | square $\frac{1}{20}$ | | | 374 | 1·14 % |
| | $\frac{1}{10}$ | | | long $\frac{1}{20}$ | | | 329 | 1·00 % |

With such small numbers the difference between the last two cannot be taken as significant, but one would expect the square plot to give a worse comparison than the long plot.

We may summarise the above results in the table below.

| Size of plot | Percentage S. D. of comparing $\frac{1}{2}$ acres | Total area required to give a S. D. of 1 % in the comparison |
|---|---|---|
| $\frac{1}{100}$th harvested as $\frac{1}{200}$ths | ·68 | ·46 acre |
| $\frac{1}{50}$ ,, ,, $\frac{1}{100}$ ,, | ·83 | ·69 ,, |
| $\frac{1}{75}$ ,, ,, $\frac{1}{150}$ ,, | ·88 | ·77* ,, |
| $\frac{1}{10}$ ,, square $\frac{1}{20}$ ,, | 1·14 | 1·30* ,, |
| $\frac{1}{10}$ ,, long $\frac{1}{20}$ ,, | 1·00 | 1·00* ,, |

* These samples are too small to give more than a rough indication of the S. D. and of the area required. I have elsewhere (*Biometrika*, vi, p. 19) given special tables for dealing with such small numbers.

The corresponding figures derived from the wheat results are set out in the second table:

| Size of plot | S. D. in lbs. of comparing two half-acres | S. D. as a % of crop on a half-acre | Total area required to give a S. D. of 1 % in the comparison |
|---|---|---|---|
| $\frac{1}{250}$th divided into $\frac{1}{500}$ths at harvest | 7·02 | ·71 | ·50 acre |
| $\frac{1}{125}$ ,,  $\frac{1}{250}$ ,, ,, | 8·54 | ·86 | ·74 ,, |
| $\frac{1}{125}$ ,,  $\frac{1}{125}$ ,, ,, | 11·56 | 1·17 | 1·37 ,, |
| $\frac{1}{25}$ ,,  $\frac{1}{50}$ ,, ,, | 10·40 | 1·05 | 1·10* ,, |
| $\frac{2}{25}$ ,,  $\frac{1}{25}$ ,, ,, | 19·40 | 1·96 | 3·84* ,, |
| $\frac{1}{100}$ taken at random | 10·28 | 1·04 | 1·08 ,, |

\* These samples are too small to give more than a rough indication of the S. D. and of the area required. I have elsewhere (*Biometrika*, VI, p. 19) given special tables for dealing with such small numbers.

Both these tables show that in the actual fields which were measured, the area of land required to give a comparison between two varieties would increase rapidly as the size of plot increased if the same accuracy were required in the result.

Roughly speaking one-twentieth acre plots of mangolds would require at least twice as much land as one-two-hundredth acre plots in order that we may place as much confidence in the result, while one-fiftieth acre plots of wheat would probably require more than twice as much as one-five-hundredth acre plots.

Hence it is clearly of advantage to use the smallest practicable size of plot.

Also the advantage of comparing adjacent plots is apparent in these examples, since with the roots less than two-thirds of the land is required to give the same accuracy as random comparison and with the wheat less than half.

Of course the comparison of whole half-acre plots would be liable to give errors of quite a different order: thus the South half-acre of mangolds is 4·7 per cent. better than the North half-acre, while the West half-acre of wheat is 8·3 per cent. better than the East half-acre; such differences would be quite impossible if the half-acres were sub-divided into the smaller sizes of plots.

STUDENT.

# "SILVER-LEAF" DISEASE.

By F. T. BROOKS, M.A.

*(Cambridge University.)*

SILVER-LEAF disease attacks several different kinds of fruit trees and various other plants. All available evidence tends to shew that this disease is becoming more and more serious each year. As the name implies, the foliage of affected plants presents a silvery appearance in contrast to the dark green colour of healthy leaves. At the commencement of attack a single branch alone usually shews signs of silvering, but in the course of a year or two the affection usually spreads throughout the tree; subsequently branches of the tree begin to die and finally the whole tree succumbs.

Percival (3) was the first to call serious attention to this disease. In 1902 he shewed that the silvery appearance of the leaves was due, not to any change in the constitution of the chlorophyll, but to the formation of air cavities in certain walls of the epidermal cells. Percival found that portions of roots of affected trees gave rise to the sporophores of *Stereum purpureum* when kept in a moist atmosphere. Upon inoculation of branches of healthy trees with portions of these sporophores, silvering of the foliage ensued. He concluded therefore that *Stereum purpureum* was the cause of the disease.

In 1906 Spencer Pickering (4) performed a number of inoculation experiments with the sporophores of this fungus, and in the large majority of cases silvering of the leaves resulted. More recently this investigator (5) has published further observations on the same disease in which he emphasises the active *rôle* played by *Stereum purpureum* in this disease. In the same publication Spencer Pickering shews that it is possible to cause silvering of Apple trees by inoculation with *Stereum purpureum* derived from Plum trees and silvering of Plum trees by inoculation with *Stereum* from a Laburnum, so it does not appear that this fungus is "specialised" to particular hosts. He mentions also the interesting fact that Plum trees occasionally recover from silvering and bear normal foliage again.

During the last three years I have been making observations and experiments in connection with this disease in Cambridgeshire, and though the work is not yet complete it has been thought desirable to publish some of the results already obtained. A preliminary account (1) of some of these observations was read at the meeting of the British Association in Sheffield in 1910. The work has been much facilitated recently by a grant which has been made to me by the Development Commissioners in order to provide fruit trees for experimental purposes; I should like to offer thanks for this assistance. I am also indebted to the Cambridge University School of Agriculture for the allocation of a plot of land at their Experimental Farm for the fruit trees provided by the grant already mentioned.

In Cambridgeshire as in other parts of the country, Plum trees are much more liable to Silver-leaf than any other kind of fruit tree. The varieties known as "Victoria" and "Czar," especially the former, are more susceptible to the disease than are other varieties. Plum trees are usually several years old before they are attacked, and I have only seen a single two-year-old tree that has become silvered without being artificially inoculated. A feature of the distribution of Silver-leaf on Plum trees is the great prevalence of the disease in small private gardens where only one or two Plum trees are present. Several plantations of Plum trees in Cambridgeshire contain more than 10 per cent. of silvered trees and in one commercial garden which has been neglected more than half the trees (Victorias) are attacked. By the kindness of Mr A. W. Hill and Mr G. Massee of the Royal Gardens, Kew, I recently had the opportunity of seeing in that district a plantation of Plum trees badly affected with Silver-leaf. In this case also more than half the Victorias were diseased and were dying rapidly.

Massee (2) notes the fact that the wild Blackthorn or Sloe sometimes becomes silvered in this country, and other species of the genus Prunus, including the Portugal Laurel, are also liable to Silver-leaf disease[1].

Nor is it only species of this genus which are liable to the malady. Apple trees are not uncommonly affected and in this district the scions of regrafted trees are often found to be silvered. Spencer Pickering (5) mentions a single Pear tree which became silvered after inoculation with *Stereum*, but I have not yet heard of Pear trees becoming attacked without such inoculation. During the discussion on Silver-leaf at the meeting of the British Association at Sheffield last year, Lieut.-Col.

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[1] I recently saw a silvered Cherry tree at Odda, Hardanger Fiord, Norway.

Prain mentioned the silvering of a Spiraea in the Royal Gardens, Kew. I have seen a few Gooseberry and Red Currant bushes affected with Silver-leaf and records have previously been published of the occurrence of the disease on Laburnum. Spencer Pickering (5) refers to a correspondent in New Zealand who makes mention of a silvered Walnut tree. Last autumn I found a Sycamore tree in the midst of a wood at Gamlingay which shewed unmistakeable signs of silvering. This tree had been cut down and the shoots arising from the stool were silvered. Mr A. W. Hill has given me information of a Horse Chestnut tree at Kew which bears silvered foliage. The tree had been lopped and shoots which subsequently developed near the wounded surface became silvered. Mr Hill has also told me of a silvered Philadelphus (commonly called Syringa) which he recently saw growing in a garden at Henley-on-Thames. It is thus seen that silvering of the foliage is a phenomenon which occurs in plants of very different relationships.

In Cambridgeshire the fructifications of *Stereum purpureum* are present in abundance on Plum trees which have died after being silvered. It is by no means rare to find a single dead branch which bears the fructifications of this fungus, the rest of the tree carrying silvered foliage. My observations shew that the sporophores appear always on dead tissues and the thought has often occurred to one that perhaps these fruit-bodies only develop after death has been caused by some other agent. However, the evidence against this view is strong, and particularly strong, I think, when the inoculation experiments to be described below are considered. It is by no means remarkable for the fructifications of such a fungus not to develop until the tissues of the host are dead; for in the case of a large number of fungi that attack trees, the spore-bodies are produced only on dead portions of the host. It sometimes takes a considerable time for the sporophores of *Stereum purpureum* to develop on the branches of silvered trees which on casual examination appear to be dead. Thus a branch which apparently dies one season may not develop fructifications until the following year; I say *apparently* dies because a branch, though externally lifeless, may still retain a large amount of living tissue; and as long as there is abundant opportunity for the fungus to invade tissues hitherto unattacked, it is easy to understand why fruit-bodies are not produced. Hence in those orchards where the wise practice of cutting out branches apparently dead is followed, but few sporophores are likely to develop.

Sporophores of *Stereum purpureum* have been seen on dead portions

of silvered Apple trees, and in the case of two of the three silvered Red Currant bushes already seen by myself this *Stereum* was growing on dead tissue at the base of the bushes.  On portions of the stool of the silvered Sycamore tree to which reference has previously been made, *Stereum purpureum* was growing, but in this connection it should be stated that similar stools of Birch close by and on which this fungus was also found bore shoots with normal foliage.

My own inoculation experiments in connection with this disease will now be described.  They may be grouped under three heads according to the material used:

(1) Inoculations with natural sporophores of *Stereum purpureum.*

(2) Inoculations with the mycelium of *Stereum purpureum* grown from spores under conditions of pure culture.

(3) Inoculations with the spores of *Stereum purpureum.*

In all cases the sporophores used either directly or for the purpose of obtaining spores had been obtained from Plum trees.


1. *Inoculations with natural sporophores of "Stereum purpureum."*

It is not necessary to describe in detail the method of these inoculations and their results, because they are identical with those of Percival and Spencer Pickering.  The inoculations were made both in old and young trees (two years old) of the Victoria and Czar varieties of Plum, pieces of damp sporophore being placed in T-shaped wounds either in side branches or in the main stem, the wounds being subsequently covered with tinfoil and bound with wool.  Almost invariably such inoculations have been followed by silvering.  Thus out of one series of 15 inoculations made in young trees last February no less than 12 resulted in silvering by the middle of May.  One characteristic of these inoculations is the profuse gumming which takes place in the neighbourhood of the wound, the masses of gum being sometimes half an inch in diameter.  However, the readiness of Plum trees to gum under injury is well known, and doubtless other organisms besides *Stereum purpureum* will induce the same reaction. If a branch is cut off a few weeks after such inoculation and examined, it is found that a part of the wood is discoloured a dark brown for a considerable distance on either side of the wound.  Sections made of the discoloured portions in the region of inoculation shew abundant hyphae in the water-conducting elements which also contain large

masses of gummy matter. The discolouration of the wood is due to the accumulation of gum in these channels. No hyphae have yet been found at the extremities of the discoloured region, so it appears that the formation of gum occurs beyond the point to which the hyphae have attained. It is noticeable that the bark immediately outside the discoloured wood of such a branch remains apparently unaffected. Though one does not wish to lay undue stress on the characters of the mycelium present in the diseased portions of the wood, one may say nevertheless that the hyphae are identical in appearance with those found in the vessels of branches of Plum trees on which fructifications of *Stereum purpureum* are growing. These hyphae are narrow, septate, and run longitudinally in the water-conducting elements of the wood; on treatment with Delafield's Haematoxylin they are characterised by the presence of small deeply-staining granules. The fact that mycelium is found both below and above the point of inoculation shews that it travels in both directions. This is of importance in connection with the spread of the disease from one branch to another. In such inoculations the first leaves to become silvered are sometimes situated a considerable distance above the wound, whereas leaves in closer proximity to it may remain normal for some time longer.

A few inoculations of the branches of a Laburnum tree with pieces of sporophore derived from Plum resulted in silvering in each case. This confirms Pickering's conclusion that in all probability there is nothing in the nature of specialised parasitism in the mode of life of this fungus.

Several series of control experiments were performed in connection with the above inoculations. In one series, wounds were made as before but nothing was inserted in them. In another, pieces of sporophore of the fungus which had been killed by boiling for 5 minutes on each of three previous days were inserted in the wounds. In other series, pieces of the fructifications of *Stereum hirsutum* and of *Polystictus hirsutus* were used instead of *Stereum purpureum*. No sign of silvering resulted in any of the trees subjected to these various kinds of treatment. The control inoculations with *Stereum hirsutum* are of particular interest because this fungus is very closely allied to *Stereum purpureum*.

A few inoculations were performed with extracts of the fructifications of *Stereum purpureum*, obtained by boiling them in water; these experiments gave negative results. However, nothing can be safely deduced from these results.

2. *Inoculations with the mycelium of "Stereum purpureum" grown from spores under conditions of pure culture.*

Soon after these investigations had been begun it was found easy to cultivate *Stereum purpureum* from spores in the same way as Marshall Ward (6) had grown *Stereum hirsutum* some years ago. Inoculations made with sporophores taken from a dead branch are not free from suspicion of contamination by some other organism, and it is with such sporophores that all previous inoculation experiments have been performed. No such objection can be raised against the use of mycelium cultivated in pure culture from spores, so this mycelium has been used for a number of experiments.

On moistening natural sporophores of *Stereum purpureum* and placing them over clean glass slides in such a way as to prevent the fructifications from coming into contact with the slides, spores are deposited in quantity and can be transferred to sterilised blocks of Plum wood arranged in suitable tubes. In a few weeks a dense mycelial growth is obtained which can be used for purposes of inoculation. The mycelium of the fungus grown in this manner is at first white in colour, but subsequently becomes tawny. Small sporophores are produced in these artificial cultures, but they lack the firmness and definite form of sporophores which develop in nature. The inoculations with the mycelium grown in pure culture were made in the same way as those in which natural sporophores were used. It should be stated that the scalpel was always sterilised before a wound was made and again before using it to transfer the mycelium from the culture tube to the wound. Last autumn six such inoculations were made in the branches of a healthy Victoria Plum tree of mature age and this spring each one of these branches was found to bear silvered foliage. Between January and March some of the young trees planted last autumn were used for a similar series of inoculations. Thus 38 inoculations were made, some in Victorias and some in Czars. By the end of May, seven of the inoculated branches on three different trees shewed silvering of the leaves, and by the middle of June this number had increased to ten. The control branches shewed no sign of silvering. As time proceeds it is highly probable that more of the inoculated branches will become silvered. The amount of the fungus placed in the wound is less when pure cultures are employed than when natural sporophores are used, and this probably accounts for the relatively smaller effect of the cultivated mycelium to date.

It is to be noted, however, that where this mycelium had the whole of the winter in which to develop, silvering resulted the following spring in every case.

One of the branches which became silvered after treatment of this kind was cut off in order to try to trace the mycelium in the tissues from the place of inoculation. In the immediate neighbourhood of the wound a cross-section shows that a sector of about 80 degrees of the wood from pith to cambium is stained a deep brown. Such wood contains numerous hyphae and large quantities of gum are present in the water-conducting elements. These hyphae are similar to those described as being present in the earlier series of inoculations. A cross-section taken about 1½ inches above the wound shows a discoloured sector of about 50 degrees, but this dark brown area does not reach quite as far as the cambium. Hyphae are found in the vessels of this region also. Three and a half inches above the wound the affected region is limited to a narrow zone immediately surrounding the pith, which also is somewhat discoloured. So far I have not been able to determine the presence of hyphae in the wood at this level. Higher still, at 6 inches above the place of inoculation, the appearance is the same as that presented at the previous level. In this region the discoloured portion passed out to a small branch which was devoid of leaves. No hyphae could be seen at this level. The portion of the stem about 3½ inches below the wound will now be described. In transverse sections the dark brown area is still found to be present but is more irregular in shape than at higher levels; hyphae are present in this discoloured zone. A lateral branch attached just above this level showed no signs of discolouration in cross-section, although it bore silvered foliage. Such was found to be the case with other lateral branches attached to the inoculated stem. It is evident from these observations that the mycelium of *Stereum purpureum* extends more rapidly in a longitudinal than in a transverse direction and that silvering of the leaves occurs at levels to which the mycelium itself has not yet reached.

A similar series of observations has been made on the branch of a young Czar Plum tree, which was inoculated with cultivated mycelium at the end of March and showed silvering of the foliage in June. In this case the discoloured zone of wood extended to a distance about 1½ inches above the point of inoculation, but silvered leaves were found at a level more than a foot above the limit of discolouration.

### 3.  *Inoculations with the spores of "Stereum purpureum."*

As far as I am aware, previous workers on this disease have made no inoculations with the spores of this fungus.  To do so is a matter of importance, because if *Stereum purpureum* is the cause of Silver-leaf, infection is probably carried by means of these spores.  During the last two years I have been making inoculations of Plum trees with the spores, but until recently with apparently no success.  Nor would it have been surprising if efforts along these lines had been baffled indefinitely, because it is a matter of considerable difficulty to cause the infection of a tree with the spores of any of the higher wood-destroying fungi.  Thus no one has yet successfully infected a tree with the spores of *Polyporus squamosus*, one of the commonest fungi of this class, and which in all probability is carried from tree to tree solely by means of spores.

After the lapse of a year, two of the twenty-one branches of fully grown Plum trees which last year were inoculated with the spores of *Stereum purpureum* now show conspicuous silvering, the successful inoculations being on two different trees.  All the uninoculated branches of these trees show no signs of silvering, so in spite of these experiments being performed by necessity in a plantation containing other silvered trees some distance away, there are good grounds for supposing that silvering in these cases followed directly from the spore inoculations.

The number of such successes as yet obtained is very small, but bearing in mind the risk of failure attached to these inoculations, and also in view of the observations recorded below, the successes are significant.

These spore inoculations were performed in the same way as the other inoculations, except that spores only were inserted into the wounds.  The spores were found to germinate fairly readily in control experiments under laboratory conditions.

One of the two branches, which already show silvering after inoculation with spores, has been cut off and investigated in the same manner as branches in which silvering has been induced by inoculation with mycelium cultivated in pure culture.  Thus at the point of inoculation, where gumming had evidently occurred, there was a zone of wood deeply discoloured occupying a position about midway between pith and cambium and covering about 90 degrees of arc.  This zone of dark brown wood was traced in a vertical plane

to levels about 4 inches above, and ¾ of an inch below, the plane of inoculation. Longitudinal sections showed the presence of the same kind of hyphae in the discoloured parts of the wood as those present in affected portions of the wood referred to in other series of inoculations. I think it may reasonably be concluded that these hyphae developed from the spores of *Stereum purpureum* originally inserted into the wound. Accumulations of gum similar to those already described were present in the water-conducting elements. Leaves 9 inches above the last trace of discoloured wood showed conspicuous silvering. On the inoculated stem, and attached between the upper and lower limits of the affected wood, a small lateral branch bore only healthy foliage. No discoloured wood was found on examining sections of this small branch.

On the young Plum trees which were planted in the experimental plot last autumn I have already performed a number of spore inoculations, but it is too early to speak of their results. The two successful spore inoculations described above had been made more than a year before silvering could be observed. But in order to see whether mycelium could be already traced in these spore inoculations of young trees one branch has been cut off and examined. At the point of inoculation a sector of dark brown wood extending over about 90 degrees was present, and in a vertical direction the zone of affected tissue stretched ¾ of an inch above, and 2 inches below, this level. Hyphae like those described above were seen in the vessels and gum was present in quantity. It is thus clear that the preliminary effects are in this case the same as in other inoculations which have eventually resulted in silvering. One may therefore expect a branch inoculated in such a manner to bear silvered foliage at a later date.

The results of all these experiments strengthen the view that *Stereum purpureum* is an active agent in inducing Silver-leaf disease in this country, and in my own mind they amount to proof that this fungus is a cause of it. It would be rash to say that *Stereum purpureum* is the *only* cause of Silver-leaf, but after considerable experience of the disease I cannot avoid the conclusion, shared of course by others, that this fungus is the agent chiefly responsible for the havoc wrought by the malady in the fruit-growing districts of this country.

The possible ways in which infection occurs in nature will now be considered. When Percival (3) first suggested that *Stereum purpureum* was the cause of the disease he considered that the roots were the

organs of the tree to be first attacked. However, in view of the distribution of the disease referred to at the beginning of this paper, and of the fact that frequently the suckers thrown up from the roots are the last parts to become silvered, it is doubtful if infection usually takes place in the roots. Furthermore, when Silver-leaf first appears in a particular area it is usually present on trees scattered here and there throughout the plantation; for several years the disease appears to spread indiscriminately, *e.g.* trees subsequently affected often bear no definite relationship in position to those originally attacked. If, on the other hand, the disease usually spread in a subterranean manner, one would naturally expect healthy trees close to silvered ones to become infected before others at a greater distance away. With Spencer Pickering, I incline rather to the view that infection more frequently occurs above ground and at some previously wounded surface. The fact that spore inoculations have been successful supports this view. Another circumstance favouring this opinion is the frequent appearance of a single silvered branch when a tree is first attacked. There is never any lack of wounds on which the spores of the fungus may germinate; pruning wounds not occluded by a callus are innumerable, and these are added to by the frequent splitting of the branches when pickers are gathering the fruit. Once the mycelium has entered the tissues it works both upwards and downwards and sooner or later enters the roots. Having entered the subterranean organs it may possibly spread through the soil to the roots of a healthy tree, but as yet there is no proof of this. Recently I saw a silvered Victoria Plum tree, nearly dead, from the bark of which at soil level a whitish mycelium was growing into the ground. It was not possible to trace this mycelium for more than a foot into the soil, and of course one cannot be certain that this mycelium belonged to *Stereum purpureum.*

A feature of the disease is the relatively long time which elapses between the first appearance of silvering and the death of the tree. This can be accounted for by the less rapid advance of the mycelium in a lateral than in a longitudinal direction; hence one side of a tree may remain healthy for some time after the other side is seriously affected. If, on the other hand, the mycelium spread rapidly in a lateral direction, all conduction of water would cease in the parts attacked, and death would soon ensue.

The occasional recovery of silvered trees has already been mentioned. I have seen a few Plum trees which, after showing conspicuous

silvering one year, have exhibited only the slightest traces of silvering the following year. This recovery may be looked upon as the victory of the host over the fungus, for until the end comes the struggle is continually being waged within the tissues of the host. But when a tree is very badly affected it is doubtful whether there is any chance of recovery. The percentage of silvered trees which regain complete health is very small and is almost negligible from the practical standpoint.

Where a single branch of a tree is silvered, endeavour should be made to cut out this limb as far back as the limit of the discoloured wood. It is useless, however, to cut away only the parts bearing silvered foliage without taking care that the branches have been severed below the lowest limit of diseased wood, for it has been shown above that the part of the wood containing the mycelium of the fungus is often a considerable distance below the foliage that is silvered. By such an operation there is a chance of saving the tree. It is obvious that where a large branch is cut away the exposed surface should be covered with a coating of tar. As soon as a silvered tree begins to die back it is advisable to remove it, for it is courting disaster to allow such a tree to remain *in situ* after death, and thereby afford opportunity for the production of the sporophores of *Stereum purpureum*. Even when trouble is taken to remove trees, they are often piled in the plantation, there to remain sufficiently long to allow of the formation of the fruit-bodies of this fungus. This practice is almost as bad as that of allowing dead trees to remain standing in the plantation. A short time ago I saw in a plantation of Plum trees a fence the posts of which were made of Plum wood. Sporophores of *Stereum purpureum* were growing in enormous numbers on these posts, and one can imagine the facility thereby afforded for the spread of Silver-leaf disease, which indeed had already made its appearance in the plantation.

The capacity of even a single sporophore of *Stereum purpureum* to produce spores is very large; furthermore, these sporophores possess great vitality, for they can be kept dry for weeks and even months, and upon being moistened will give rise to spores again within a few hours. The sporophores of *Stereum purpureum* in this country are to be found all the year round. The fungus is such a common saprophyte that it is impossible to eradicate it, but every means should be taken along the lines indicated above to prevent it from producing spores in fruit plantations.

At an early date I hope to publish another paper giving fuller details of the action of the mycelium of *Stereum purpureum* on Plum wood. Investigations are also being made upon the *modus operandi* of silvering and upon possible means of curative treatment, though I am afraid the outlook for the latter does not appear to be very promising at present.

### SUMMARY.

· 1. The distribution of Silver-leaf disease is described.

2. Inoculations of branches of Plum trees with sporophores of *Stereum purpureum* have almost invariably resulted in silvering.

3. The mycelium of *Stereum purpureum* has been cultivated in pure culture from spores and inoculations with this mycelium have caused silvering.

4. A small number of inoculations with the spores of *Stereum purpureum* have resulted in silvering.

5. Various series of control experiments have given negative results.

6. The investigations strengthen the view that *Stereum purpureum* is the active agent which causes Silver-leaf disease in this country.

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# THE FORMATION OF CALCIUM CARBONATE IN THE SOIL BY BACTERIA.

### By C. T. GIMINGHAM, F.I.C.

#### (*University of Bristol.*)

THE important part played by calcium carbonate in maintaining the fertility of the soil makes it of interest to investigate all the causes which determine both its formation and decomposition. A number of processes which are continually going forward in the soil tend to diminish the stock of calcium carbonate present; yet, in spite of this, and without frequent additions of chalk or lime from other sources, most soils, under normal conditions, remain more or less in a state of fertility. An equilibrium is set up by which the withdrawal of calcium carbonate is balanced by the results of another set of actions which restore base to the soil. This enables many soils which contain only very small quantities of lime to retain their neutral reaction and so to produce fair crops.

Hall and Miller (*Proc. Roy. Soc.* B, Vol. 77, 1905) have shown that one of the actions which tend to maintain the supply of base is the oxidation, to the state of carbonate, of the calcium salts of organic acids such as oxalic, known to be formed in considerable quantities in plant tissues[1], and by moulds and bacteria. Soil organisms are responsible for this action. Calcium oxalate is the most abundant of such salts and at Mr A. D. Hall's suggestion this work was begun with the object of finding out the nature of the organisms concerned in the conversion of this substance into calcium carbonate in the soil.

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[1] In this connection it is interesting to note that the loss of calcium carbonate from the farmyard manure plot of Broadbalk wheat field at Rothamsted is considerably less per acre per annum than from the unmanured plot. This seems to be due to the conversion of organic calcium salts in the dung into carbonate by the action of micro-organisms.

A few early references to the fermentation of oxalates are of interest. In 1876 Stützer (*Ber.* 9. 1395), experimenting with plants in $CO_2$-free air and growing in artificial soils containing calcium oxalate or tartrate, came to the conclusion that they were capable of utilising these organic substances directly as food. However, later, Schmöger (*Ber.* 12. 753), repeating these experiments, proved that the growth observed was due to the carbon dioxide formed by the oxidation of the oxalate or tartrate, and that when the air was kept quite free from this carbon dioxide, the amount of growth was very slight.

Munro (*J.C.S.* 1886, 49. 648) states that alkaline oxalates are rapidly attacked by "soil ferments" and are available as base in nitrification experiments. Finally, in the *Proc. Univ. Durham Phil. Soc.* Vol. II. pt. 3, Prof. Potter claims to have isolated an *anaerobic* schizomycete possessing the power of converting oxalate to carbonate, but details are not given.

*Experimental.*

Hall and Miller's experiments were repeated showing that organisms capable of fermenting calcium oxalate to carbonate, are present in the soil. A solution of the following composition was employed:

| | | | | | |
|---|---|---|---|---|---|
| Sodium chloride | .. | ... | ... | 1 gm. | |
| Ammonium sulphate | | ... | ... | 1 ,, | in 1000 c.c. |
| Dihydrogen pot. phosphate | | ... | 0·5 ,, | Tap water |
| Magnesium sulphate | | ... | ... | 0·25 ,, | |
| Glucose | | | | | |

The small quantity of glucose was added in order to provide a little soluble organic matter to assist in starting growth.

50 c.c. quantities of this solution plus a weighed amount—usually about 0·2 gm.—of calcium oxalate[1] and inoculated with 0·5 gm. soil were placed in an incubator at 25° C. At the end of a variable time (from 3—5 weeks) it was found that most of the oxalate had disappeared and had been replaced by calcium carbonate in the form of small crystals mostly sticking to the bottom and sides of the flask.

The amount of oxalate remaining was determined by means of a permanganate solution. The following experiment will illustrate the kind of results obtained:

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[1] The calcium oxalate used in all the experiments was powdered fine enough to pass through a sieve with 100 meshes to the inch.

*Three flasks as above each seeded with 0·5 gm. soil.*

|      | Nov. 8 | Dec. 1 | Dec. 19 | Dec. 30 |                         |    |    |
|------|--------|--------|---------|---------|-------------------------|----|----|
| A$_1$ | 0·208  | 0·067  |         |         | gms. CaC$_2$O$_4$ remaining |    |    |
| A$_2$ | 0·21   |        | Trace   |         | ,,                      | ,, | ,, |
| A$_3$ | 0·21   |        |         | none    | ,,                      | ,, | ,, |

Mr A. Amos, B.A., had previously attempted to get various species of bacteria to cause this oxidation of calcium oxalate in pure culture, but among those he employed only Azotobacter chroococcum was found capable of producing any carbonate.

Further experiments in this direction were made; and a large number of bacteria, yeasts and moulds were tested in pure culture but none appeared able to decompose the oxalate. There was usually very poor growth of the organism in the ammoniacal nutrient solution; and a number of species, among them Azotobacter, refused to grow at all. In such cases, a small quantity of sterilised calcium oxalate was introduced into a culture of the organism already growing strongly in a favourable medium and the flask allowed to remain in the incubator for a long period. In the Azotobacter cultures, after about four months, some of the oxalate was utilised and carbonate crystals appeared: this however was not until all soluble organic matter had been used up. None of the other organisms were found to attack the oxalate at all.

The following is the list of those grown in presence of calcium oxalate and showing no power of decomposing it:—B. aceti, xylinum, Kützingianum, Pasteurianum, ascendens, rancens, acetosum, aerogenes, acidi lactici, butyricus, prodigiosus, coli communis, mycoides, subtilis, fluorescens liquefaciens, megatherium, Proteus vulgaris, Sarcina lutea, Saccharomyces (several species), Dematium, Mycoderma, Oidium lactis, Aspergillus glaucus, Mucor mucedo, Penicillium spp.

Difficulty was frequently experienced in obtaining a good end-point in the titration of the oxalate by means of permanganate when there was much accumulation of organic matter owing to an abundant growth of the organism. It was found more convenient in such cases to make determinations of the carbonate formed, in duplicate flasks. The method employed was that recommended by Amos (*Jour. Agric. Sci.* Vol. I. p. 322) for the estimation of carbonate in soils.

Attempts to isolate an organism from the soil responsible for the change were at first unsuccessful. Various nutrient media were employed and a large number of types of bacteria isolated and

subcultured. None of them had the power of decomposing calcium oxalate. Better results however were obtained when, instead of the ammoniacal nutrient solution, a clear water extract of soil was used— 100 gms. soil to 1 litre of water. In this solution the action proceeded much more rapidly. By simply adding a small quantity of finely powdered calcium oxalate to 50 c.c. of this soil-extract (unsterilised) and placing it in an incubator at 25° C., crystals of calcium carbonate appeared in the flask in less than three weeks.

The results were similar whether the extract was prepared from a somewhat stiff loam or from a light sandy soil, the production of carbonate being however more rapid in the case of the sand, which was extremely poor in organic matter. The lack of much soluble organic matter seems to throw the organisms back on the oxalate for their supply of carbon.

In some experiments toluene or chloroform was added to the solution. Under these conditions there was no trace of carbonate formation in six weeks, whereas control flasks without antiseptic produced crystals in sixteen days. The action was therefore not enzymic.

A dilution method was employed to purify such cultures. Soil-extract in which carbonate had been formed from oxalate was filtered under sterile conditions into new flasks containing sterile soil-extract plus calcium oxalate; and when carbonate crystals appeared in this second lot, the filtration etc. was repeated. This process of dilution was carried out five or six times until in the final set of flasks the change was quite rapid, requiring only 8 or 10 days for the formation of carbonate crystals.

Plates were made from these solutions in ordinary gelatine or agar nutrient media and in the majority of cases only one, two or three types of colonies developed. These were subcultured on various media, the organisms obtained pure, and their power of oxidising oxalate in pure culture tested by somewhat heavy inoculations into sterile soil-extract containing calcium oxalate in suspension. Some of these pure cultures were found to be capable of forming carbonate and eventually six types of bacteria were isolated possessing this power. Three of these came from Rothamsted Park soil and three from the soil of the College Farm at Wye. The rate at which any one of these organisms would produce carbonate crystals from calcium oxalate varied considerably at different times, even in the same soil-extract, and the kind of medium on which the organism had been previously grown apparently had some influence. A subculture from a flask in which the action

had already begun, usually—though not always—showed an increased rate of production of carbonate up to a certain limit. The following Table gives the number of days which elapsed in different experiments with these organisms in pure culture, before it was possible to distinguish carbonate crystals in the flask. These are compared with the time required by a mixed culture consisting simply of unsterilised soil-extract to which calcium oxalate had been added, and which thus contained many kinds of soil organisms. It will be seen that the impure mixed culture on the whole was more active in causing the fermentation of the oxalate than any of the pure cultures.

| Organism | I | II | III |
|---|---|---|---|
| | Days | Days | Days |
| Ra | 29 | 18 | 14 |
| R₂b | 54 | 23 | |
| Rd | 54 | | |
| W₂a | 30 | 17 | 10 |
| Wb | 26 | 17 | 12 |
| Wx | 65 | | |
| Impure culture | 15 | 11 | 8 |

The presence of oxygen is essential for the formation of carbonate. It was not found possible to get any action, either with a mixed culture, or with any one of the bacteria in pure culture, under anaerobic conditions; and Prof. Potter's observation (*loc. cit.*) could not be confirmed. The crystals of calcium carbonate appeared first always at the edge of the culture solution where the surface of the liquid touched the glass.

It seems probable that a number of bacteria in the soil are able to oxidise calcium oxalate, if driven to it by lack of other food; and probably more types could have been isolated. In these circumstances, it is perhaps unnecessary to give any special description of the particular organisms referred to.

This work was carried out partly in the laboratory of the Lawes Agricultural Trust at Rothamsted and partly at the S. E. Agricultural College, Wye. The author wishes to express his great indebtedness to Mr A. D. Hall for his kind interest and advice throughout.

# THE FAT GLOBULES OF MILK IN RELATION TO CHURNING.

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## PART I.

It is a matter of common experience in dairy work, that different creams vary in their behaviour during the operation of churning; and many theories have been adduced to explain the factors which possibly affect, adversely or otherwise, what may be termed, for want of a more suitable word, *churnability*[1].

Not only may the creams of the various breeds show marked difference in behaviour, but those of cows of the same breed may frequently exhibit individual peculiarities.

In attempts to find the cause or causes of this variation, inquiries have usually been directed to the two most obvious variable factors; (*a*) the size of the fat globules, (*b*) the constitution of the milk serum.

*Von Leeuwenhoek*, in 1697, was the first to observe that milk is not a homogeneous fluid, but that it consists of globules of fat suspended in a serum.

*Schroeder* (1872) showed that when milk is fractionally creamed, the butter fat obtained from the first fraction, has a lower melting point and lower specific gravity than the butter fat obtained from the last fraction. He also stated that the latter contained no butyric acid. (The first fractions should contain the larger sized globules.)

*Sturtevant* (1880) observed that the size of the globules is affected by the changes which influence the cow; for instance, bran and sharps

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[1] Woll, 1889, uses the term "Churnability of the milk fat" for what is termed the "Butter Ratio," *i.e.* the ratio of butter to the original milk.

diminish the size.   He points out that the relative size of the globules varies with the period of lactation, the globules becoming more uniform as lactation progresses.   He also says, but without any proof in support of the statement, that Jersey milk has a larger average-sized globule than has Ayrshire milk ; and that in the latter, there is less uniformity in size, and a greater proportion of the smaller globules.

Sturtevant states that " Churning is a physical process and acts upon the larger globules only.   Hence of two milks showing like figures to analysis, the churn will separate more butter from the one than from the other, especially if the milks be from two distinct breeds of cows."

*D'Hont* (1890)[1] published an account of some investigations on the *size* of the globule and gives excellent photogravures ; he says, " if, in addition to taking the milk from one herd, the milk of cows of different breeds is examined, the fat globules from cows of the same breed are seen to be fairly regular in size, which differs with each breed."

In accordance with this, the breeds are divided into 3 classes.

    1.   Breeds with small globules.
    2.   Breeds with medium globules.
    3.   Breeds with large globules.

All his results, however, are based on purely visual examination of the photographs, without any measurements.   He mentions that he was obliged to give long exposures ; so that the smaller globules would not be recorded, because they are continually moving.

D'Hont points out a fact of great importance in actual dairying practice, *i.e.* that a milk containing large globules will give a more complete separation of the fat, whether the separation be spontaneous or mechanical.

He draws attention, also, to the fact that the size of the globules will have a great effect on the churning; that when the globules are large, the churning will be rapid ; and that it is advantageous to add to milk containing medium or small sized globules a certain quantity of milk with large globules.   He points out that this method is actually practised in England, where many dairies keep Jersey cows, in the proportion of one Jersey to each eight cows of other breeds, in order to increase the yield of butter ; " though, doubtless, most makers do not surmise the cause of the increase."

[1] D'Hont's original paper is not generally available; and Lloyd in his abstract gives no indication as to how or why D'Hont made his classification.   The authors have acquired a copy of his original paper which will be presented to the Royal Agricultural Society.

He mentions, also, that not only is the churning more rapid with cream having large sized globules; but, as the large globules entangle the smaller ones in the process of aggregating the globules, that is in churning, therefore a milk having large globules will give a better yield of butter than a milk containing the same amount of fat, but having smaller globules.

D'Hont states that food has no influence on the size of the globules. He points out that, for the manufacture of cheese, a breed with more small globules would give a better result than one with large globules.

*Collier* (1891) gives the result of a large number of determinations. He found that the size diminished as the period of lactation advanced; that is, that the relative number of large globules diminished and the smaller globules increased; so that in the last quarter of the period of lactation there was 89 % more fat globules than in the first quarter of the period.

*Woll* (1889, 1890, and 1892) came to the same conclusion and he states that morning milk has more large globules than evening milk.

*Hall* (1891) confirms D'Hont's results as to the effect of the larger size of the globule in the Jersey cow, though he gives no experimental data in support of his statements. He also says that " it is well to avoid all milk with large globules, for this industry"—the manufacture of cheese.

*Schnellenberger* (1893) confirmed these results. He, also, estimated the number and size of fat globules of milks of different breeds.

*Kluseman* (1893) fractionally separated the globules by using a Laval separator, working at different temperatures and different speeds. He worked on the individual milk of five cows, and on the mixed milk of seven. He subjected the fractionated globules to various investigations, and came to the following conclusions:

| | |
|---|---|
| Melting Point.<br>Freezing Point.<br>Insoluble Fatty Acids.<br>M.P. of Fatty Acids.<br>F.P. of Fatty Acids. | Higher in the small than in the large globules. |
| Volatile Acids.<br>Iodine Number. | Lower in the small than in the large globules. |

*Gutzeit* (1895) published the results of a very detailed investigation on the same subject, working on the milk from a large number of cows. He collected the large globules by allowing the milk to stand for six

hours and skimming off the cream; and the small, by separating off
and discarding the intermediate cream, then coagulating the skimmed
milk and separating the small globules.

He determined the specific gravity, the melting point, the re-
fractive index, the insoluble fatty acids, the volatile fatty acids, the
saponification number, the iodine absorption number, of the butter-fat
—from each sized globules. His conclusions were that the fat globules
of all sizes have the same chemico-physical composition.

He made most careful and accurate counts on the milks of many
cows, continuing his observations during the whole period of lactation;
and he gives a very detailed account of the method used (Babcock's)
with explanatory figures.

*Lemus* (1902) separated the large and small globules by interrupted
milking, the first milk being taken to represent the small and the last
milk the large globules—a method which would appear to be very
inexact. He states that, in the majority of cases, the smaller globules
contained more oleic acid and less volatile acids than did the larger
ones.

*Woods and Bitting* (1905, p. 25)[1] mention a point of essential
interest, in that they state that "the milk from certain breeds, as
Jersey and Guernsey, is characterised by large globules, while that of
other breeds, as Ayrshire and Holstein, *usually* contain small globules.
*There is, however, a wide individual difference in all breeds, Jersey
sometimes producing small globules and Holstein large ones.*" This is
confirmed by the authors, as will be seen from an examination of their
figures.

*Woods* gives some measurements of globules, recording globules of
a size never seen by us, as shown by the following:

| Size | 4 μ | 6 μ | 8 μ | 12 μ | 16 μ | 20 μ | 24 μ | 28 μ |
|---|---|---|---|---|---|---|---|---|
| Number of each size per 1000 globules | 95 | 220 | 427 | 152 | 67 | 20 | 10 | 9 |

*Shaw and Eckles* (1909) point out that Kluseman worked on too
small a number of cows; and that, apart from this, the variations in his
results do not warrant his conclusions. They further state that Lemus'
method is inexact, as it does not give a real separation; and moreover,

[1] This and the paper by Shaw and Eckles, contain the best information on the fat
globules and their interrelation with the peculiarities in milk.

that instead of the relative size of the fat globules being invariably larger in the "stripper" milk than in the "fore" milk, sometimes the reverse is true.

The much debated question as to the presence of a mucous envelope round the globules, is one of considerable importance in the problem of churnability. This envelope, the membrane of Acherson, was supposed to be derived from the protoplasm of the cell. It is most generally accepted that no such membrane exists; yet Storch (1897) conducted a series of experiments which tend to show that there is such a mucous envelope. Also Sturtevant, Abderhalden and Völtz and others assert that there is a membrane.

*Woods and Bitting* (1905, p. 26) state that "it is now considered that milk is a natural emulsion and that what appears to be a membrane is not different from what is seen in other emulsions, having the fat similarly divided."

Storch shows that this last statement is not quite correct; since the fat cannot be extracted by simply shaking with a solvent. The authors hold no particular view, not feeling satisfied with the evidence on either side. But as the solution of this point is one of essential importance to the problem before us, Storch's work is being repeated and other experiments are being made. If an envelope does surround the globules, however slight it may be, then the aspect of the problem of churnability would be altered considerably.

The process of the formation of the fat globules of milk in the udder should throw some light on the problem of relative churnability. Our knowledge of this, however, is in an unsatisfactory state, and the reader is referred to a paper by Woods and Bitting for a short account of what is known on this point. A full description of the mammary gland in the cow, and its physiology, is given by Grimmer (1910).

In the following pages, the results of experimental observations made in 1909 and 1910 are communicated. The opportunity for an inquiry of this nature is most readily obtained at one or other of the agricultural shows, where samples of milk from cows of many different breeds are available, at the same time and under similar conditions. Unfortunately, the abnormal conditions, which obtain in the show-yard, affect the cows, and, consequently, the constitution of the milk; hence it is not a matter of surprise that the analytical results obtained do not always conform to average figures.

Some preliminary work was carried out at the Royal Agricultural Society's Show, held at Gloucester in June, 1909, a report on the results

being published in Mr Ernest Mathews' article—*Dairy Cattle and the Butter Test*. In 1910, through the courtesy of the Council of the Royal Agricultural Society, greater facilities were given for a continuation of this inquiry. Experimental churning was carried out in the show-yard at Liverpool, samples being sent to this laboratory for physical examination and chemical analysis.

All possible precautions were taken to obtain representative samples; but, at the same time, the necessity of dispatching these without delay made it impossible to use the mixed morning and evening milks; so that samples were taken from the morning milkings throughout.

Each sample, after the addition of four drops of formalin (40 %) as a preservative, was poured into a 6 oz. bottle, so as to fill it completely, leaving no bubble of air. This precaution is very necessary to prevent partial churning of the contents in transit[1].


## PART II.

### *Determinaton of the Fat Globules.*

Up to the present, only one method has been used, as far as the authors can ascertain, namely, that of Babcock. This consists of intro-ducing the milk into a capillary tube, sealing the ends with vaseline[2], and examining the tube, immersed in glycerine, under the microscope. Babcock's paper (1885) is inaccessible in this country, but a very full account of the method is given by Gutzeit (1895), together with an exposition of the optical properties of a capillary tube and corrections to be applied.

In this method, the globules are allowed to rise, so as to lie under the upper curved surface of the capillary tube, placed horizontally, and the *number* of globules in a definite volume is measured. From this

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[1] The corking of a bottle entirely filled with liquid is facilitated by the insertion of a loop of string down the side of the cork, which allows a means of exit for the superfluous contents, the string being withdrawn after the cork has been pushed home.

It is very necessary that care should be taken in the transmission of milk samples. The photographs taken in the year 1909 show the effect of sending milk samples in bottles not completely filled, which allows a certain amount of churning to take place. Pl. III. (photo. 17) shows such a churning. This may be compared with Pl. I. (photo. 3). In the one the globules have collected together into groups: in the other this has not taken place.

D'Hont's photographs show the effect of insufficient precautions being taken against incipient churning.

[2] Gutzeit sealed the ends of his tubes by holding them in a small gas flame.

figure, the percentage of fat in the milk being known, the *average volume* of the fat globule is calculated.

This method gives the average volume of the fat globules, but does not take into account the disposition of the fat globules of various sizes. It was expected that more light would be thrown on the subject if one were to make an actual measurement of each globule, so as to ascertain whether the average volume was the average of a wide or of a narrow range of variations in volume.

To measure each globule by Babcock's method is impossible, for several reasons; in the first place the smallest globules all show Brownian movement; again, the globules are not all in one plane; also, there is no means of checking off the globules which have been counted. To overcome these and other difficulties, photomicrographs of each sample of milk were prepared in the following manner.

It was not found necessary to dilute the milk. The contents of the sample bottle were well agitated to ensure thorough mixing, and a drop was transferred by means of a platinum loop, to the counting chamber of a Thoma-Zeiss Haemocytometer. This apparatus was slightly modified from the form used in blood examinations, inasmuch as the chamber had a depth of 0·015 mm. instead of the usual 0·100 mm. The opacity of a film of milk of greater thickness than 0·015 mm. makes this modification essential. The usual ruling was retained, the field being divided into squares of 0·0025 sq. mm. area.

On account of the tendency of the fat globules to rise, the preparation must be photographed in a horizontal position; and to obviate blurring of the image, due to Brownian movement, the exposure must be short. One-fiftieth of a second was found to be sufficiently rapid. The magnification, in all cases, was 500 diameters.

It was found that the ruling of the cell was indistinct or quite invisible through the film of milk; on this account, therefore, it was photographed separately at the same magnification as the milk, and for the enumeration and measurement, a transparency of the ruling was superimposed on the photomicrograph of the milk. Photomicrographs of the different samples are reproduced in photos. 1—16, Pls. I.—III.

For the measuring of the diameter of the globules, a transparency was made of a series of circles, the smallest being exactly 500 $\mu$ in diameter and each differing from the next smallest by 500 $\mu$ in diameter: so that the diameters of each circle would correspond exactly to the photograph, the smallest circle representing a globule of 1 $\mu$ in diameter magnified 500 times.

Taking the photomicrograph, with the ruling of the cell superimposed upon it, the transparency of circles is placed upon any globule, so that the circle coincides with the periphery of the globule. This gives the measurement of the globule. The diameter is noted down, and a pencil mark made upon the globule, which enables the enumerator to count at his leisure, and which ensures that all globules shall be measured and that none shall be measured twice.

The fat globules in an area of 16 squares on each photomicrograph were enumerated and measured. The results are shown in Tables A, B and C.

The mass of fat contained in the total number of globules of each particular size was calculated from these figures, and the percentage of fat, by weight and by volume, was then computed. A comparison of these results with the figures representing the percentage of fat by weight, determined by chemical analysis, is given in Tables A 2, B 2, C 2.

These show the agreement between the physical and chemical determinations to be, on the whole, reasonably close; the discrepancies which occur, may be due to an inaccurate sample taken for examination or to experimental error; any inaccuracy in measurement or counting being multiplied 1,666·6 times in the calculation.

Tables A 1 and A 2 give the results of determinations made on four breeds of cows, the milks from all the cows being well mixed before separating.

In order to investigate the individual variation in cows of the same breed, enumerations were made on individual Shorthorn and Jersey cows, and the results are given in Tables B and C.

Tables A, B, and C give, also, the total number of globules per cubic millimeter. It will be seen that the variation in number is very great; but this confirms the results of other workers.

It seems that our method, though far more laborious than Gutzeit's or Babcock's, is more accurate, in that one cannot miss the small globules, of $1\,\mu$ diameter, which are so difficult to determine on account of their movement, and the omission of which makes a great difference in the results[1].

Again, in Babcock's method there is no means of checking off the accuracy of the count, and of the sample taken up by the capillary tube. In our method, however, the actual volume of fat in each globule is

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[1] For example in milk 3151—a milk with comparatively few small globules—the mean diameter calculated from the figures given is $3\cdot07\,\mu$. If the globules of $1\mu$ diameter are omitted, the diameter is $3\cdot73\,\mu$.

TABLE A 1. *Measurements of four breeds mixed milks.*

| | Breed | Number of cows from which milk was obtained | Number of Globules in 0·0006 mm.³ | | | | | | | | | | | | No. of globules per mm.³ | % of Fat (by volume) in each size globule | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | 11 μ | 10 μ | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ | Total | | 11 μ | 10 μ | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ |
| 16. | Shorthorn | 83 | — | 1 | — | 1 | 5 | 10 | 29 | 97 | 213 | 295 | 456 | 1107 | 1,845,000 | — | ·09 | — | ·04 | ·15 | ·19 | ·32 | ·54 | ·50 | ·21 | ·04 |
| 17. | South Devon | 7 | — | — | — | — | 2 | 20 | 45 | 184 | 403 | 500 | 757 | 1911 | 3,185,000 | — | — | — | — | ·60 | ·38 | ·49 | 1·03 | ·95 | ·35 | ·07 |
| 18. | Jersey | 52 | — | — | 1 | 6 | 26 | 44 | 65 | 160 | 211 | 199 | 432 | 1144 | 1,906,000 | — | — | ·06 | ·27 | ·78 | ·83 | ·71 | ·89 | ·49 | ·14 | ·04 |
| 19. | Red Poll | 10 | 1 | 1 | 1 | — | 15 | 41 | 57 | 155 | 255 | 423 | 601 | 1550 | 2,054,000 | ·12 | ·09 | ·06 | — | ·45 | ·77 | ·62 | ·87 | ·60 | ·29 | ·05 |

TABLE A 2.

| Total % of Fat | | | | |
| --- | --- | --- | --- | --- |
| | Calculation | | Analytical | Difference Calculated and Analytical Results |
| | By volume | By weight | By weight | |
| | 2·08 | 1·98 | 2·60 | − 0·72 |
| | 3·32 | 3·00 | 3·64 | − 0·64 |
| | 4·21 | 3·8 | 3·83 | − 0·03 |
| | 3·92 | 3·54 | 3·28 | + 0·26 |

TABLE B1.  *Shorthorn individual milks.*

| Sample | Number of Globules in 0·0006 mm.³ | | | | | | | | | | No. of globules per mm.³ | % Fat by volume in each size globule | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ | Total | | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ |
| 669 | — | 3 | 5 | 39 | 52 | 120 | 189 | 279 | 522 | 1209 | 2,015,000 | — | ·13 | ·15 | ·73 | ·56 | ·67 | ·45 | ·19 | ·05 |
| 199 | — | 1 | 3 | 24 | 69 | 300 | 554 | 637 | 881 | 2469 | 4,115,000 | — | ·04 | ·09 | ·45 | ·75 | 1·67 | 1·43 | ·44 | ·08 |
| 979 | 3 | 22 | 42 | 68 | 64 | 118 | 152 | 207 | 363 | 1039 | 1,732,000 | 3·4 | ·98 | 1·26 | 1·28 | ·70 | ·66 | ·36 | ·14 | ·03 |
| 1001 | 1 | 10 | 37 | 55 | 58 | 101 | 144 | 229 | 273 | 908 | 1,514,000 | 1·5 | ·45 | 1·11 | 1·04 | ·63 | ·56 | ·34 | ·16 | ·02 |
| 5101 | — | — | 13 | 47 | 74 | 210 | 466 | 413 | 556 | 1779 | 2,966,000 | — | — | ·39 | ·89 | ·90 | 1·17 | 1·10 | ·29 | ·05 |
| 279 | — | 1 | 18 | 28 | 46 | 161 | 312 | 428 | 844 | 1838 | 3,064,000 | — | ·04 | ·54 | ·53 | ·50 | ·90 | ·74 | ·30 | ·07 |

(Shorthorns)

TABLE B2.

| Total % of Fat | | | Difference Calculated and Analytical Results |
|---|---|---|---|
| Calculation | | Analytical | |
| By volume | By weight | By weight | |
| 2·94 | 2·64 | 2·21 | + ·43 |
| 4·96 | 4·46 | 2·54 | ÷1·92 |
| 5·60 | 5·04 | 3·03 | ÷2·01 |
| 4·37 | 3·93 | 3·23 | + ·70 |
| 4·78 | 4·30 | 3·45 | + ·85 |
| 3·62 | 3·26 | 2·87 | + ·39 |

TABLE C1.  *Guernsey individual milks.*

| Sample | Number of Globules in 0·0006 mm.³ | | | | | | | | | | % Fat by volume in each size globule | | | | | | | | | No. of globules per mm.³ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ | Total | 9 μ | 8 μ | 7 μ | 6 μ | 5 μ | 4 μ | 3 μ | 2 μ | 1 μ | |
| Guernseys 3151 | — | — | 5 | 51 | 73 | 143 | 189 | 163 | 270 | 894 | — | — | ·15 | ·96 | ·81 | ·80 | ·44 | ·11 | ·02 | 1,490,000 |
| 7051 | — | 1 | 8 | 40 | 70 | 214 | 364 | 365 | 407 | 1469 | — | ·04 | ·24 | ·75 | ·76 | 1·19 | ·86 | ·25 | ·03 | 2,449,000 |
| 0151 | — | 5 | 26 | 77 | 87 | 117 | 133 | 147 | 320 | 912 | — | ·22 | ·78 | 1·45 | ·95 | ·65 | ·31 | ·10 | ·03 | 1,520,000 |
| 2151 | — | 1 | 2 | 20 | 57 | 221 | 411 | 335 | 409 | 1456 | — | ·04 | ·06 | ·38 | ·62 | 1·23 | ·97 | ·23 | ·04 | 2,428,000 |

TABLE C2.

| Total % of Fat | | | Difference Calculated and Analytical Results |
|---|---|---|---|
| Calculation | | Analytical | |
| By volume | By weight | By weight | |
| 3·29 | 2·95 | 2·82 | +·13 |
| 4·14 | 3·72 | 3·51 | +·21 |
| 4·50 | 4·05 | 3·65 | +·40 |
| 3·58 | 3·21 | 4·15 | −·94 |

ascertained, and from this the percentage of fat by weight is calculated[1]; this should agree with the figures obtained by chemical analysis, and any difference between them is a measure of the error, either in taking the drop for the chamber, or in counting.    If the error is large, any experiments carried out with that milk can be omitted ; or the photo-micrograph can be measured again.    For example, our determinations on milks 199 and 979 are incorrect, probably due to taking an incorrect sample for the determination.

### Graphic Representation.

Curves were drawn to represent graphically the results of these determinations of the fat globules (Figs. 1 to 3).    In both curves, the
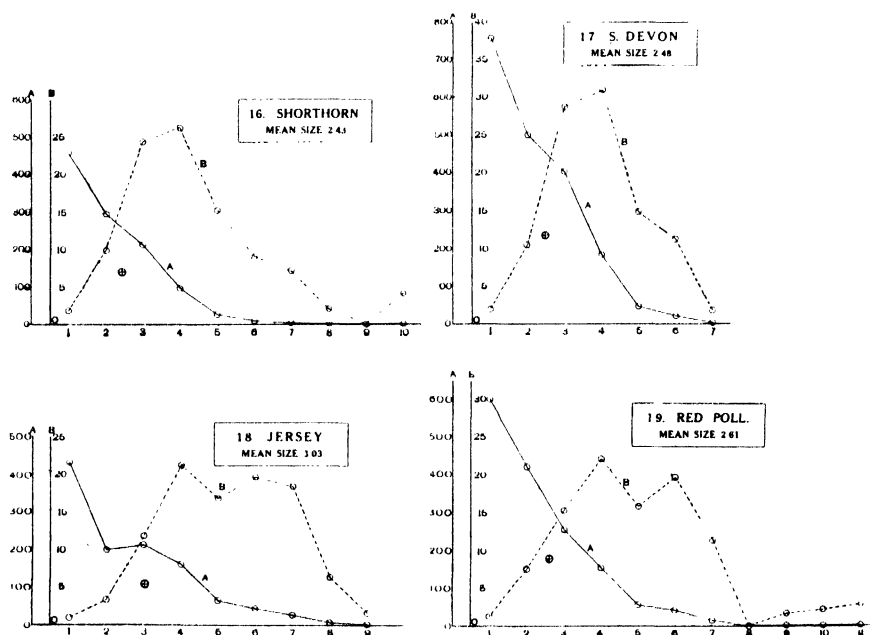


Fig. 1.    Mixed milk of four breeds.

sizes of globules are plotted as abscissae.    In curve $A$, the number of globules of each size is plotted as ordinates ; in curve $B$, the percentage of fat contained in each size of globule.    Two curves, $A$ and $B$, have been drawn for each of the milks of which measurements have been

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[1] In our calculations, the sp. gr. of fat was taken as ·930 and of milk as 1·034 ; as given in Allen (1898), Vol. IV., p. 158.

MEAN SIZE 2.50
Shorthorn
Sample 279.

MEAN SIZE 2.58
Shorthorn
Sample 199.

MEAN SIZE 2.65
Shorthorn
Sample 669.

MEAN SIZE 2.78
Shorthorn
Sample 5101.

MEAN SIZE 3.16
Shorthorn
Sample 1001.

MEAN SIZE 3.20
Shorthorn
Sample 979.
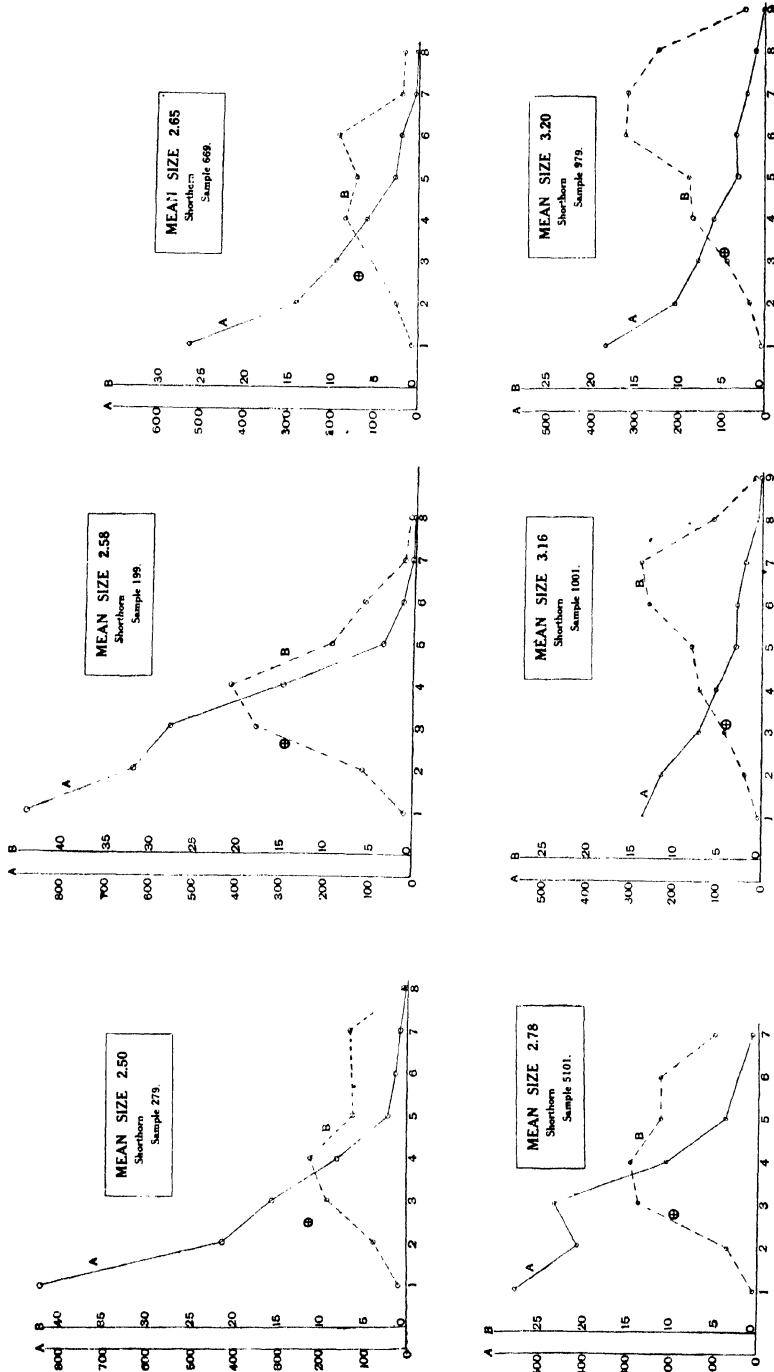
Fig. 2.  Shorthorn individual milk.

made and photographs taken; reference and comparison can be made by means of the sample number.

The centres of gravity of curves $A$ were taken. Vertical and horizontal lines cutting the abscissae and ordinates, drawn through the centres of gravity, give the *mean size* of the globules, on the one hand, and the *mean number*, on the other hand. These are given in the first two columns of Table D.
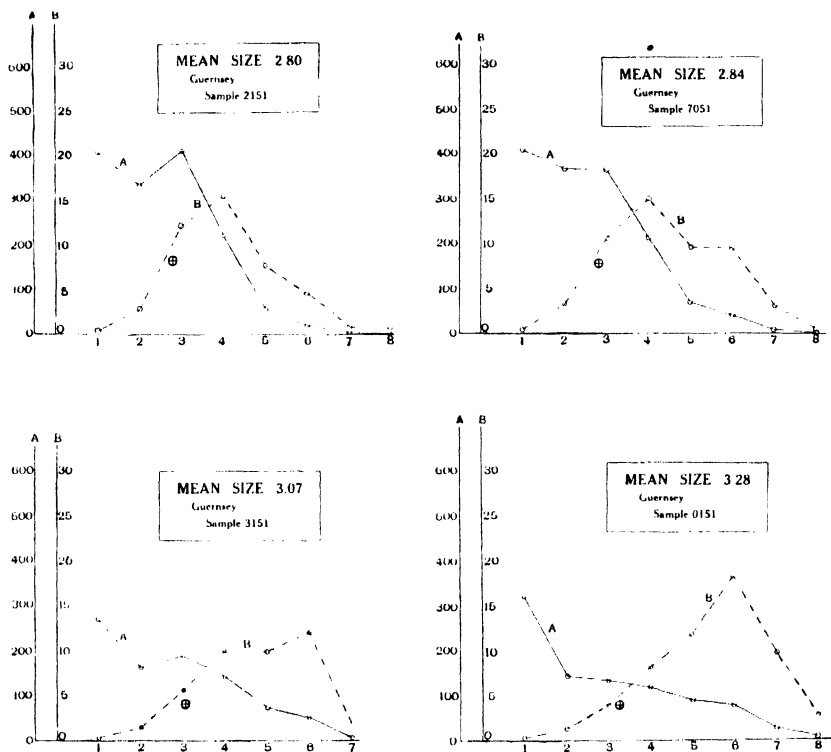


FIG. 3.   Guernsey individual milk.

From these figures can be calculated the percentage of fat which would be contained in the average number of globules of the average size. This has been done and the results are given in column 3 of Table D. The results obtained by actual measurements and by analytical methods are given in the next two columns, for the purposes of comparison.

It will be seen that the figures agree fairly well, considering the very large possibility of error in any method where such an excessively small quantity of milk is taken for the initial measurement.

One might classify the milks according to the mean size of the globules; arbitrary numbers would have to be taken and it would not be inconvenient to divide them into four classes:

Milks with globules of small mean size below $2 \cdot 50 \mu$,

|  | ,, | ,, | ,, | medium | ,, | from $2 \cdot 50$ to $2 \cdot 75 \mu$, |
|--|----|----|----|--------|----|---------|
|  | ,, | ,, | ,, | large | ,, | ,, $2 \cdot 75$ to $3 \cdot 00 \mu$, |
|  | ,, | ,, | ,, | very large | ,, | above $3 \cdot 00 \mu$. |

TABLE D.　*Table of mean sizes.*

|  | Mean | | Mean % fat, by vol. | Measured % fat by vol. | Analytical % fat, by wt. | Ratios | | |
|---|---|---|---|---|---|---|---|---|
| | Size | Number | | | | $\dfrac{\text{Proteid}}{\text{Fat}} \times 100$ | $\dfrac{\text{Proteid}}{\text{Lactose}} \times 100$ | $\dfrac{\text{Fat}}{\text{Lactose}} \times 100$ |
| INDIVIDUAL | | | | | | | | |
| Shorthorn | | | | | | | | |
| 669 | 2·65 | 137 | 2·23 | 2·94 | 2·21 | 128·0 | 65·97 | 51·51 |
| 279 | 2·50 | 223 | 3·14 | 3·62 | 2·87 | 101·0 | 60·80 | 60·17 |
| 979 | 3·20 | 93 | 2·66 | 5·60 | 3·03 | 103 6 | 65·15 | 62·87 |
| 199 | 2·58 | 294 | 4·41 | 4·96 | 2·54 | 129 5 | 65·15 | 50·29 |
| 1001 | 3·16 | 82 | 2·06 | 4·37 | 3·23 | 102·2 | 69·18 | 67·72 |
| 5101 | 2·78 | 194 | 5·76 | 4·78 | 3·45 | 83·49 | 57·03 | 68·31 |
| Guernsey | | | | | | | | |
| 7051 | 2 84 | 157 | 3·11 | 4·14 | 2·82 | 99·42 | 72·41 | 72·83 |
| 0151 | 3·28 | 79 | 2·45 | 4·50 | 2·65 | 92·32 | 72·16 | 78·16 |
| 2151 | 2·80 | 166 | 3·18 | 3·57 | 4·15 | 78·32 | 67·72 | 86·48 |
| 3151 | 3·07 | 82 | 2·07 | 3·29 | 2·82 | 114·6 | 66·74 | 58·26 |
| MIXED | | | | | | | | |
| Jersey (18) | 3·03 | 107 | 2·60 | 4·21 | 3·83 | 78 07 | 63·75 | 81·66 |
| Red Poll (19) | 2·61 | 179 | 2·79 | 3·92 | 3·28 | 96·65 | 65·64 | 67·92 |
| South Devon (17) | 2·48 | 236 | 3·07 | 3·32 | 3·62 | 81·08 | 66·82 | 82·41 |
| Shorthorn (16) | 2·43 | 141 | 1·77 | 2·07 | 2·60 | 100·8 | 63·90 | 63·42 |

The authors suggest this classification, as being the first to be based upon actual measurements of the globules. D'Hont's classification was based upon a purely visual examination of the photographs, and it is obvious from a comparison of Tables A, B, C with the photographs that one cannot form a correct estimate in such a manner.

Supposing that this basis of classification is taken, it will be seen that, comparing the figures obtained for the six individual Shorthorn cows, some contain small, some large mean globules; and one contains very large mean globules. From this, it would appear that the individual

variation is so great as to make such a system of classification impossible; but it has been observed by other investigators, as well as by ourselves, that the size of the globules in individual cows is influenced by a number of factors. Woods, for instance, states that Jersey milk *usually* contains large, and Holstein milk small globules; but that the variation in individual cows of the same breed is very great, so that Jersey milk *may* contain small, and Holstein milk large globules. It has been shown, more particularly by Gutzeit, that the mean size of the globules is influenced by such factors as the period of lactation, the feeding, the manner in which the cow is milked, etc.

Gutzeit also found a great variation in cows of the same breed, for instance, he gives the following (1895, p. 648):

| Breed | Maximum Diam. $\mu$ | Minimum Diam. $\mu$ |
|---|---|---|
| Jersey ... ... | 4·21 | 2·81 |
| Angler... ... | 3·60 | 2·36 |
| Shorthorn ... | — | — |
| Montavoner ... | 3·87 | 2·00 |
| Hollander ... | 3·47 | 1·99 |
| Britenburger ... | 3·29 | 1·99 |

The variation in the size of globules of individual cows, dependent on the period of lactation, is shown by an examination of his table on p. 561, as follows:

| Lactation period in months | Cow 1 | | Cow 3 | | Cow 9 | |
|---|---|---|---|---|---|---|
| | Mean vol. from table $\mu^3$ | Mean Diam. calc. $\mu$ | Mean vol. from table $\mu^3$ | Mean Diam. calc. $\mu$ | Mean vol. from table $\mu^3$ | Mean Diam. calc. $\mu$ |
| 1st | 19·6 | 3·3 | 19·4 | 3·3 | 18·6 | 3·3 |
| 3rd | 15·6 | 3·1 | 17·8 | 3·2 | 15·2 | 3·1 |
| 5th | 12·6 | 2·9 | 10·5 | 2·7 | 11·3 | 2·8 |
| 7th | 11·8 | 2·8 | 12·2 | 2·9 | 11·9 | 2·8 |
| 9th | 10·3 | 2·7 | 10·0 | 2·7 | 11·8 | 2·8 |
| 11th | 9·3 | 2·6 | 9·6 | 2·6 | 11·1 | 2·8 |

It would appear, therefore, that the results obtained so far are in accordance with the observations of others, and that the system suggested is not misleading.

It is obvious, however, that for the purpose of ascertaining the variation in churnability—the primary object of our work—each cream must be considered as being obtained, not from a milk of any particular breed, *but from a milk having a particular mean size of globule, without any respect to the breed*: also that it is necessary to compare *the churnability of milks containing different mean sized globules,* rather than milks of different breeds; that is to say that Shorthorn milk 669 is comparable with Red Poll 19, and Shorthorn milk 279 is comparable with South Devon 17.

The method adopted by the authors of plotting curves, etc., to obtain the mean size of globule, is laborious, but it shows the distribution of the fat in different sizes of globules. It was considered desirable to investigate this, in order to ascertain what difference there might be in different milks: this could not be done by using Babcock's method.

To obtain the volume of the mean globule, it is only necessary to count the *number* of globules in a definite area of the photograph: the depth of the cell, and the fat content of the milk (by analytical results) being known, the volume, or the diameter of the mean globule, can be calculated, but more accurately than in Babcock's method.

### The Constitution of the Milk Serum.

That the various constituents of the milk may play an important part in the question of churnability has already been pointed out. Solutions of casein have marked emulsifying properties, and the large quantity of this substance present in milk serum, at once suggests a possible factor which might affect churnability. In a lesser degree, the other constituents, such as lact-albumen, lactose, etc., must be considered. In the light of modern ideas regarding colloid chemistry, it must not be forgotten that the mineral salts present in milk serum may have an influence which is out of all proportion to their relative mass.

The facts concerning these problems, embodied in the present communication, unfortunately fail to lead to a definite conclusion, on account of the impossibility of making a direct comparison between analytical determinations and the results of churnability experiments on identical milks. Preliminary work on a means for the experimental determination of churnability, has given promise of a solution of these difficulties (*vide* Churning Experiments, below).

The following figures were obtained, with the object of establishing a relationship between the size of the fat globules and the varying proportions of serum constituents. A fairly complete chemical analysis of the same samples used for the physical determinations was made, the generally accepted methods being used throughout. From the figures thus obtained, the ratios of Fat : Proteid, Proteid : Lactose, and Fat : Lactose were worked out and are embodied in Tables E and F.

It will be seen that there is no correlation of these ratios, and a possible explanation of this may be the fact that the milks of too small a number of cows, for *average* purposes, were used (six of one breed and four of another, respectively). A comparison of these analytical results with the measurements made on the same milks, throws no more light on the subject : they are added, however, to Table D.

At the bottom of each table are given the corresponding figures for the mixed milk of each breed. These figures are seen to be abnormal; this may be accounted for by the fact that the samples were taken during the milk trials, when, one may suppose, the condition of the cows was abnormal.

## Churning Experiments.

Hitherto, practical experience in the dairy has been the only means of determining the precise moment at which the butter " comes " in churning ; the term *churnability*, therefore, is merely arbitrary ; and experimental determinations based on such observations are purely empirical.

As a means of determining the churnability of a cream, a self-recording experimental churn was devised. For this purpose, the " end-over-end " principle being impracticable, a modification of the well-known " plunger " churn was adopted. The churn consists of a tall, cylindrical, glass vessel (gas jar) in which a plunger rises and falls, and communicates its movements by means of levers to a pencil which records these movements on a travelling band of paper. The mechanical details of the machine are so contrived as to allow the plunger to fall freely by its own weight through the cream in the churn, the raising of the plunger being a direct mechanical lift. By reference to the diagram (Fig. 4) it is seen that the plunger rod $A$ is surrounded by a sliding sleeve $B$, to which an up and down motion is communicated by the actuating rod $C$. The plunger moves freely in the sliding sleeve, but is prevented from falling through it by the nut at the top ; so that it

TABLE E. *Chemical Analyses.*

*Individual Guernsey Milks*

| Cow | Ash By Analysis | Ash Variation from Average | Total Solids By Analysis | Total Solids Variation from Average | Total Solids 2+7+11+13 | Total Solids Variation from Analytical Results | Fat By Analysis | Fat Variation from Average | Solids not Fat By Analysis | Solids not Fat Variation from Average | Proteid By Analysis | Proteid Variation from Average | Lactose By Analysis | Lactose Variation from Average | Water By diff. = 100% − 4 | Water Variation from Average | Ratios Fat : Lactose | Variation from Average | Ratios Proteid : Fat | Variation from Average | Ratios Proteid : Lactose | Variation from Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 6a | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 3151 | ·79 | +·01 | 11·75 | −·75 | 11·68 | −·07 | 2·82 | −·71 | 8·93 | −·04 | 3·23 | −·11 | 4·84 | +·06 | 88·25 | +·75 | 58·26 | −15·67 | 114·6 | +18·44 | 66·74 | −3·02 |
| 0151 | ·77 | −·01 | 12·66 | +·16 | 12·46 | −·20 | 3·65 | +·12 | 9·01 | +·04 | 3·37 | +·03 | 4·67 | −·11 | 87·34 | −·16 | 78·16 | +4·23 | 92·32 | −3·84 | 72·16 | +2·40 |
| 7051 | ·83 | +·05 | 12·54 | +·04 | 12·65 | +·11 | 3·51 | −·02 | 9·03 | +·06 | 3·49 | +·15 | 4·82 | +·04 | 87·46 | −·04 | 72·83 | −1·10 | 99·42 | +3·26 | 72·41 | +2·65 |
| 2151 | ·75 | −·03 | 13·04 | +·54 | 12·95 | −·09 | 4·15 | +·62 | 8·89 | −·08 | 3·25 | −·09 | 4·80 | +·02 | 86·96 | −·54 | 86·48 | +12·55 | 78·32 | −17·84 | 67·72 | −2·04 |
| Average | ·78 | | 12·50 | | | | 3·53 | | 8·97 | | 3·34 | | 4·78 | | 87·50 | | 73·93 | | 96·16 | | 69·76 | |
| Mixed Guern. Milk | ·67 | | 11·71 | | | | 3·67 | | 8·04 | | 2·98 | | 4·46 | | 88·29 | | 82·30 | | 81·19 | | 66·82 | |

TABLE F.  *Chemical Analyses.*

*Individual Shorthorn Milks*

| Cow | Ash | | Total Solids | | | | Fat | | Solids not Fat | | Proteid | | Lactose | | Water | | Ratios | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | | | | | | | | | | | | | Fat : Lactose | | Proteid : Fat | | Proteid : Lactose | |
| | By Analysis | Average Variation from | By Analysis | Average Variation from | By Addition of Results 2+7+11+13 | Variation from Analytical Results | By Analysis | Average Variation from | By Analysis | Average Variation from | By Analysis | Average Variation from | By Analysis | Average Variation from | By diff. = 100% − 4 | Average Variation from | | Average Variation from | | Average Variation from | | Average Variation from |
| 1 | 2 | 3 | 4 | 5 | 6 | 6a | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 5101 | 0·76 | − ·01 | 12·15 | + ·53 | 12·14 | − ·01 | 3·45 | + ·56 | 8·70 | − ·03 | 2·88 | − ·18 | 5·05 | + ·26 | 87·85 | − ·53 | 68·31 | + 8·16 | 83·49 | − 24·4 | 5·03 | − 6·85 |
| 1001 | 0·77 | 0 | 12·05 | + ·43 | 12·07 | + ·02 | 3·23 | + ·34 | 8·82 | + ·09 | 3·30 | + ·24 | 4·77 | − ·02 | 87·95 | − ·43 | 67·72 | + 7·57 | 102·2 | − 5·7 | 69·18 | + 5·30 |
| 669 | 0·75 | − ·02 | 10·64 | − ·98 | 10·08 | − ·56 | 2·21 | − ·68 | 8·43 | − ·30 | 2·83 | − ·23 | 4·29 | − ·50 | 89·36 | + ·98 | 51·51 | − 8·64 | 128·0 | + 20·1 | 65·97 | + 2·09 |
| 199 | 0·72 | − ·05 | 11·65 | + ·03 | 11·60 | − ·05 | 2·54 | − ·35 | 9·11 | + ·38 | 3·20 | + ·23 | 5·05 | + ·26 | 88·35 | − ·03 | 50·29 | − 9·86 | 129·5 | + 21·6 | 65·15 | + 1·27 |
| 979 | 0·79 | + ·02 | 11·81 | + ·19 | 11·78 | − ·03 | 3·03 | + ·14 | 8·78 | + ·05 | 3·14 | + ·08 | 4·82 | + ·03 | 88·19 | − ·19 | 62·87 | + 2·72 | 103·6 | − 4·3 | 65·15 | + 1·27 |
| 279 | 0·81 | + ·04 | 11·41 | − ·21 | 11·35 | − ·06 | 2·87 | − ·02 | 8·54 | − ·19 | 2·90 | − ·16 | 4·77 | − ·02 | 88·50 | + ·21 | 60·17 | + ·02 | 101·0 | − 6·9 | 60·80 | − 3·08 |
| Average | 0·77 | | 11·62 | | 11·50 | − ·12 | 2·89 | | 8·73 | | 3·06 | | 4·79 | | 88·38 | | 60·15 | | 107·9 | | 63·88 | |
| Mixed Shorthorn Milk | ·62 | | 10·4 | | 9·94 | − ·5 | 2·6 | | 7·80 | | 2·62 | | 4·10 | | 89·6 | | 81·66 | | 100·8 | | 63·9 | |

falls freely through the sleeve in its downward motion; but, in the upward motion of the sliding sleeve, it is forcibly lifted. The small spring shown in the figure is introduced to minimise jar. The lower extremity of the plunger rod carries a pair of semicircular hinged vanes, which in the downward motion of the plunger through the cream, are opened out flat, but which, in the up-stroke, fall together and offer a minimum resistance to the lifting of the plunger through the cream.
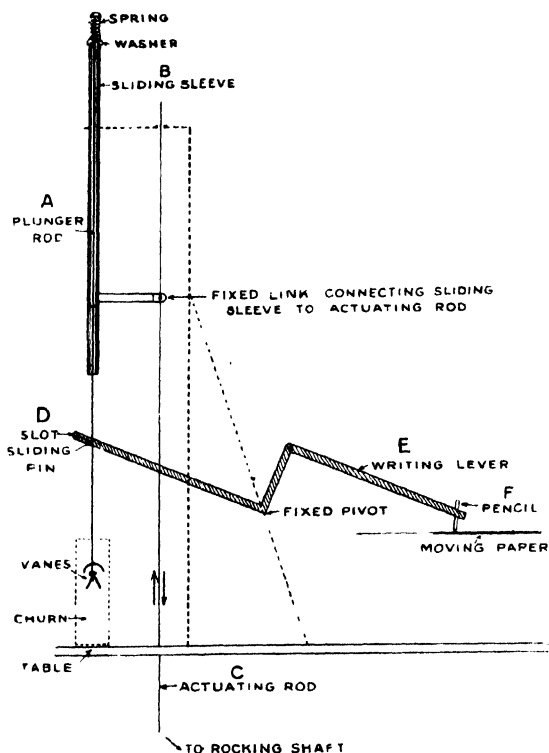


FIG. 4. Diagram of machine.

The motions of the plunger are transmitted by means of a sliding pin working in the slotted lever *D*, through the writing lever *E* to the recording pencil *F*.

In the complete machine, six such experimental churns are arranged side by side, in order that simultaneous determinations may be made.

In an actual experiment, the glass jar is about two-thirds filled with cream, thinned, if necessary, with water. The glass jar is then

placed in a larger vessel (see Fig. 5) filled with water at the desired
temperature of churning.   The speed of the machine and the weight of
the plunger rod must be so adjusted, that the time occupied by the free
fall of the plunger just allows the vanes to reach almost to the bottom
of the jar, before being drawn up by the sliding sleeve.   Each stroke
of the plunger is marked as a line on the moving roll of paper, and the
length of this line is a measure of the viscosity or the " thickness " of
the contents of the churn.   As churning proceeds and the cream thickens,
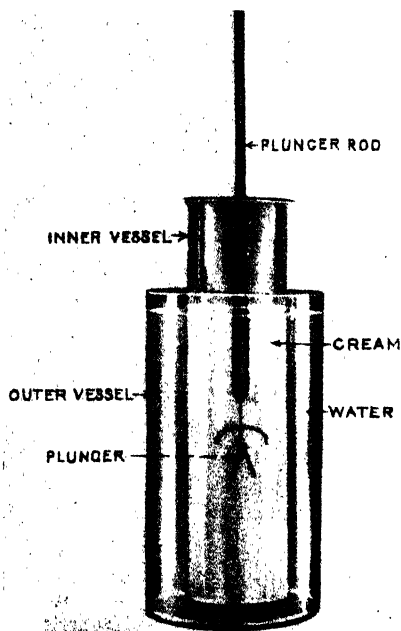


Fig. 5.   Plunger inside inner and outer vessel.

the plunger meets a greater resistance in its down-stroke ; and, the
time allowed for each cycle being the same, it naturally follows that
the lines become shorter and shorter.   When the butter is formed, the
plunger, in its collapsed up-stroke position, is drawn through the mass;
but it is arrested in the ensuing down-stroke, by the impinging of
the open vanes on the top of the butter, so that the stroke is then
very short.

On the diagram traced by the pencil, the extremities of the strokes
are joined by a line which forms a continuous " curve."   The point at

which a sudden drop appears in the curve indicates the moment of formation of butter (see Fig. 6). If the cream is too thick at the commencement, it assumes a "sleepy" condition during churning, and no progress is made.

Numerous trials of the apparatus have demonstrated its practicability and the following diagram is a copy of a tracing, made in actual experimental churning. Unfortunately, the first opportunity of giving the apparatus a practical trial was that offered at Liverpool, in June, 1910, and the greater part of the time was occupied in ascertaining the most suitable conditions for comparative determinations. Further determinations must be made, before any final conclusions can be drawn.
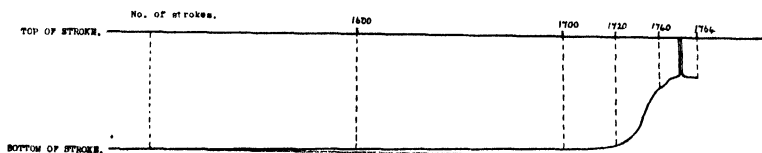


FIG. 6. A normal curve obtained with the "churnability" apparatus. During 1500 strokes no alteration could be detected. After that, the cream became more viscous —"thick"—up to the 1720th stroke. During the next 20 strokes the butter "came," the point of transition being very near the 1730th stroke.

### The Optimum Temperature for Churning.

The importance of churning at the correct temperature, is generally recognised by dairy workers. In actual practice, the rule seems to be, that churning is commenced at two or three degrees below the optimum temperature, so as to make allowance for the rise in temperature due to mechanical heating. The question of temperature being of such importance, the following experiment was carried out, with a view to the determination of optimum conditions.

An iron frame, fitted to contain six glass jars (8 ins. high, $2\frac{1}{2}$ ins. diameter) is fixed on pivots in a wooden vat, the whole frame with its contents being capable of rotation on a horizontal axis (Figs. 7 and 8), thus forming a series of "end-over-end" churns. The vat is filled with water which is maintained constantly at the temperature at which it is desired to churn. The level of the water is such, that when the frame is in a position with the mouths of the jars uppermost, they are raised about two inches above the surface. Into each jar is poured six ounces of cream, previously diluted to contain 30 % of fat. The covers of the jars are adjusted and fixed and the frame is then rotated. As each

sample of cream is completely churned, the apparatus is stopped, and
that particular jar is removed, after which the churning of the remainder
is resumed, the operation being repeated until all the samples under
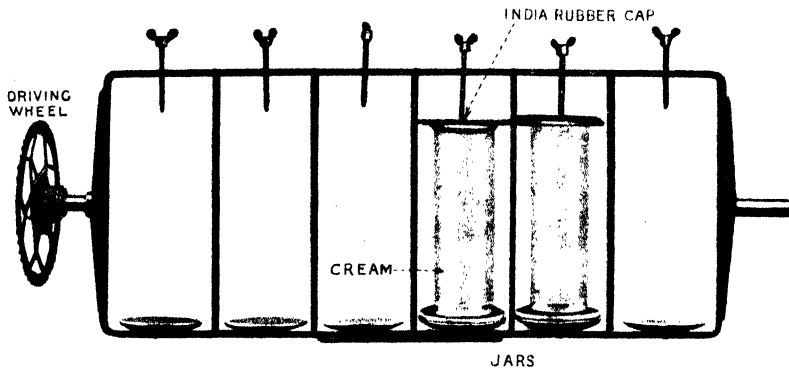


Fig. 7.   Iron frame with six partitions, driving wheel, and jars.
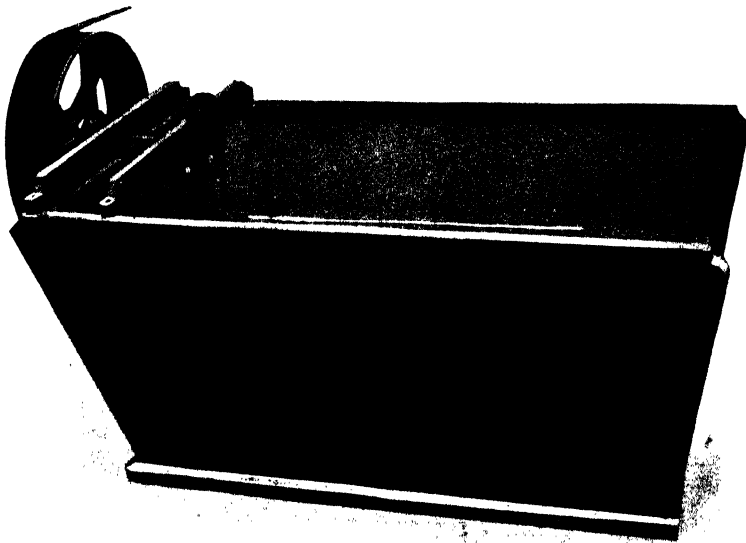


Fig. 8.   Tub containing the frame.

investigation are churned.   The butter formed in each jar is strained
off and weighed, and the percentage of fat remaining in the butter-milk
is estimated.

In the experiments at Liverpool, samples of cream from six different breeds were churned in this apparatus, at 54°, 59°, and 64° Fah. respectively. From the figures obtained, the amount of fat originally taken being known, the percentage converted into butter was calculated.

Considerable differences in behaviour are exhibited by the different breeds, but further work must be carried out, before absolute values can be stated.

In conclusion, it is obvious from the results obtained, that until a large number of complete determinations on different samples of milk have been made, no progress towards a solution of the problems of churnability is possible. This preliminary work has laid the foundation for further investigation, which, it is hoped, will lead to definite conclusions.

The question of the relation of size of the fat globules to churnability has, so far as the authors are aware, been considered on purely chemical and physical grounds, and the physiological aspect of the case appears to have been entirely ignored. From a consideration of the physiological process of the secretion of fat in the living cell, it would appear that the size of the fat globules is determined, once and for all, at the time of their discharge from the secreting cells. If this surmise is correct, the inhibitory action of the emulsifying constituents of the serum in the de-emulsification of the cream, should be the clue for subsequent investigation.

The authors are much indebted to Mr Ernest Mathews for assistance in this work.

The blocks for Figs. 5 to 8 are reproduced by permission of the Council of the Royal Agricultural Society of England.

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## DESCRIPTION OF PLATES.

The small numbers to the right, below each photomicrograph, refer to the sample number of the milks, of which analytical data is given in the text. The determination of the globules, in each case, is to be found in Tables A to D and Figs. 1 to 3 ; a correct estimation and interpretation of the photographs can only be arrived at by comparison with these tables and figures.

Of the actual area measured only a small portion is shown, sufficient to show the general characters of each sample.

All photographs of milks are at a magnification of 500 diameters.

## PLATE I.

*Mixed milks of different breeds* × 500.

| No. 1. | Jersey Milk... | ... | ... | ... | ... | Mean diam. | | 3·03 |
|--------|----------------|-----|-----|-----|-----|------------|------|------|
| No. 2. | Red Poll ... | ... | ... | ... | ... | ,, | ,, | 2·61 |
| No. 3. | Ayrshire | | | | | | | |
| No. 4. | South Devon | ... | ... | ... | ... | ,, | ,, | 2·48 |
| No. 5. | Shorthorn ... | ... | .. | ... | ... | ,, | ,, | 2·43 |
| No. 6. | Guernsey | | | | | | | |

## PLATE II.

*Individual Shorthorn Milks* × 500.

Each from an individual cow. A comparison of these photographs with each other, and with the tables and figs., illustrates the great variation in individual milks from cows of the same breed, and supports the conclusions on p. 166.

| No. 7. | Period of Lactation. | 61 days ... | ... | Mean diam. | | 2·78 |
|--------|----------------------|-------------|-----|------------|------|------|
| No. 8. | ,, | ,, | 59 days ... | ... | ,, | ,, | 3·16 |
| No. 9. | ,, | ,, | 48 days ... | ... | ,, | ,, | 2·65 |
| No. 10. | ,, | ,, | 69 days ... | ... | ,, | ,, | 2·58 |
| No. 11. | ,, | ,, | 60 days ... | ... | ,, | ,, | 3·20 |
| No. 12. | ,, | ,, | 62 days ... | ... | ,, | ,, | 2·50 |

## PLATE III.

*Individual Guernsey Milks* × 500.

| No. 13. | Period of Lactation. | 48 days ... | ... | Mean diam. | | 3·07 |
|---------|----------------------|-------------|-----|------------|------|------|
| No. 14. | ,, | ,, | 25 days ... | ... | ,, | ,, | 3·28 |
| No. 15. | ,, | ,, | 70 days ... | ... | ,, | ,, | 2·84 |
| No. 16. | ,, | ,, | 174 days (6 months) | | ,, | ,, | 2·80 |

These photographs also accentuate the conclusions on p. 166. They also confirm Gutzeit's conclusions; it is to be noticed that the milk from the cow which had been in milk for 25 days has very few small globules; whereas that from the cow which had been in milk for 6 months has few large ones.

No. 17. *Mixed Ayrshire Milk;* photograph made in 1909. This shows the incipient churning, or aggregation of the globules, due to the fact that the sample-bottle of milk was subjected to some shaking during transmission.

This may be compared with No. 3, Pl. I., in which case the sample-bottle was filled quite full.

No. 18. *Jersey Milk separated.* Content of fat ·01 %. A few very small globules can be seen, but no large ones. It serves to show the ruling, and the comparative size of the squares.

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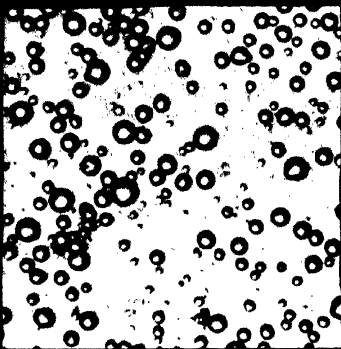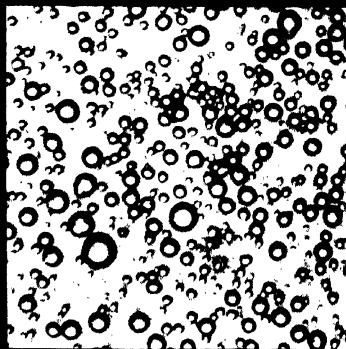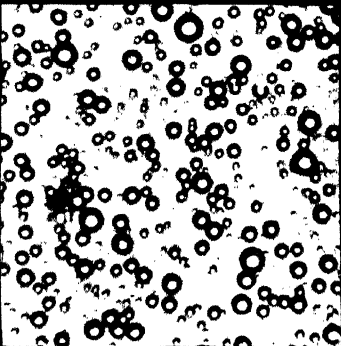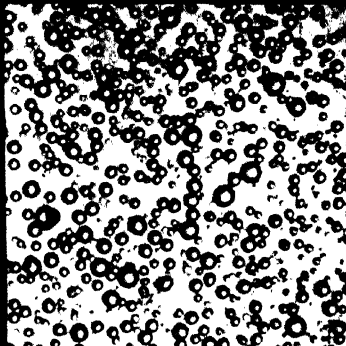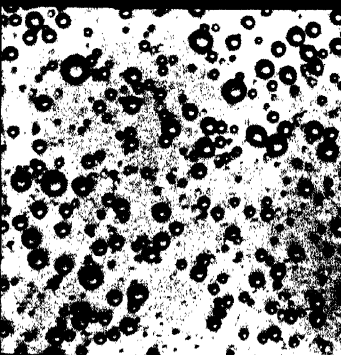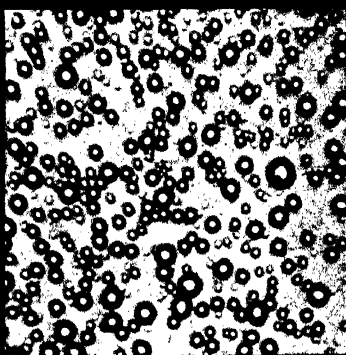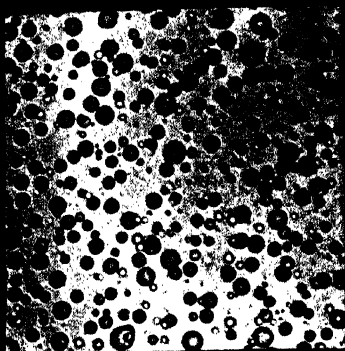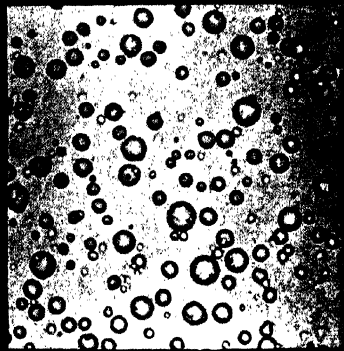
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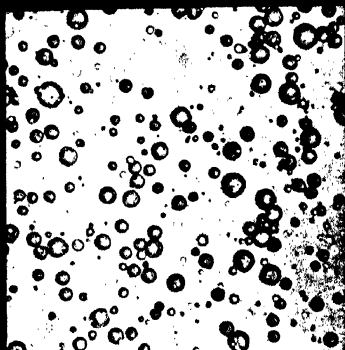
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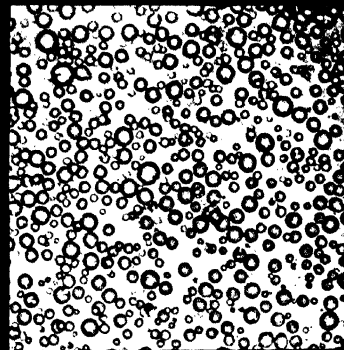
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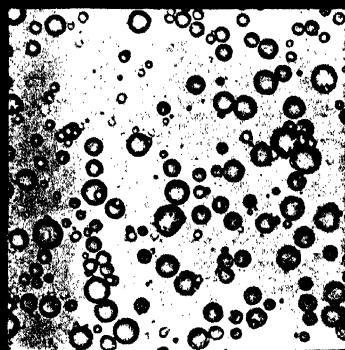
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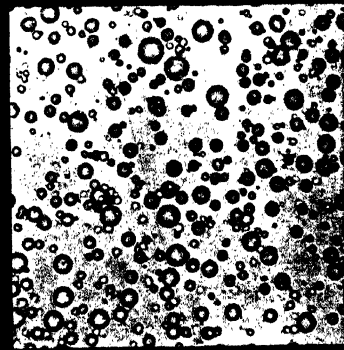
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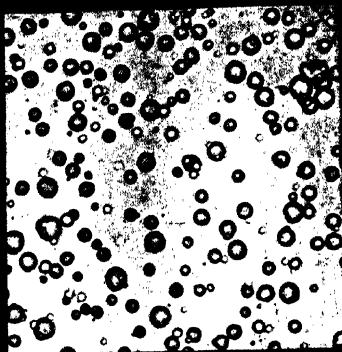
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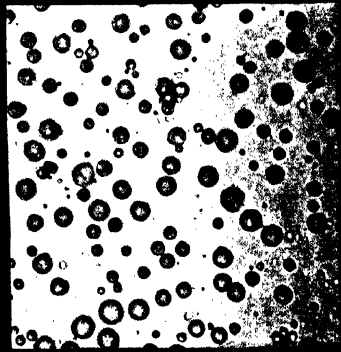
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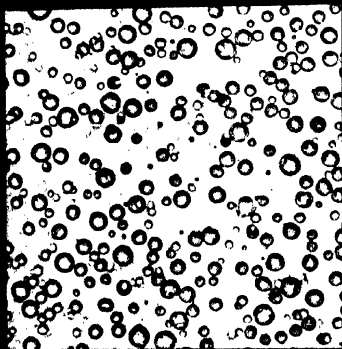
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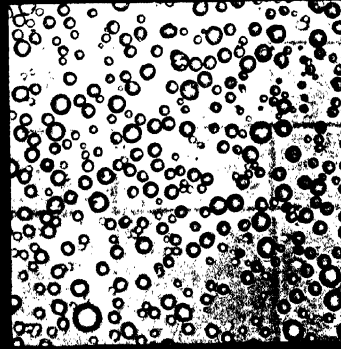
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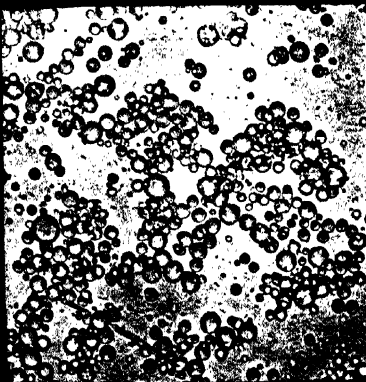
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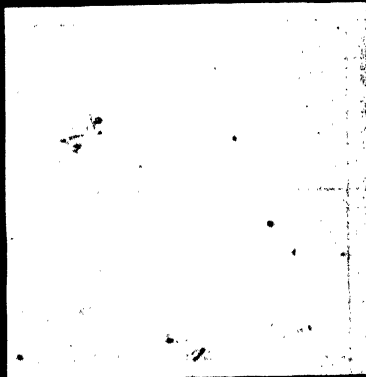
No. 18

# OXIDATION OF ARSENITES TO ARSENATES IN CATTLE-DIPPING TANKS.

By W. F. COOPER, B.A., F.C.S., and G. A. FREAK, B.Sc., A.I.C.

*From the Cooper Laboratory for Economic Research.*

THE gradual loss of efficiency of certain alkaline arsenite dipping baths has been traced by Brünnich to the oxidation of the arsenite to arsenate (Australian Association for the Advancement of Science, 1909). In this latter form, arsenic is not nearly so efficacious for the eradication of ticks, as in the reduced condition.

In countries possessing warm climates (U.S.A., South America, South Africa, Queensland, etc.) where cattle raising forms one of the chief industries, it is necessary to immerse the cattle periodically in a bath containing sodium arsenite as one of its chief constituents. Hence, this question of the oxidation of the arsenites to the less useful arsenates is one of great importance. Brünnich showed that this oxidation was accelerated by the presence of tar products; and further, that Stockholm tar induced oxidation more readily than carbolic acid. This latter fact has such an important bearing on the composition of an ideal dipping fluid that the authors considered it desirable to compare the relative effects of wood tar and commercial cresylic acids.

For this purpose, solutions of sodium arsenite, containing the tar products emulsified by means of soap, were placed in bottles, and air was drawn through them for prolonged periods. It was not feasible to measure the volume of air, but it was sucked through, daily, for several weeks. The first three experiments were made in the winter, the bottle being placed on the bench : solutions Nos. 4 and 5 were exposed to direct sunlight in order to imitate, as far as possible, the conditions obtaining in actual dipping in hot climates. The quantities of sodium arsenite and arsenate were determined in each solution before and after the passage of air.

The method of analysis was as follows :—

A definite volume of the solution was acidified with dilute sulphuric acid to precipitate the fatty acids from the soap; these fatty acids were then filtered off through a wet filter. The filtrate was warmed to about 70° C. and completely extracted with hot benzene, in order to remove the remaining organic matter; the aqueous solution was freed from dissolved benzene by boiling, and was then cooled. Two equal volumes were taken; in one, the arsenate was determined directly by the addition of ammonia, and magnesia mixture containing a large percentage of ammonium chloride; in the other, the arsenic was completely converted, by oxidation with nitric acid, to arsenate which was determined by the magnesium pyroarsenate method as before.

The magnesium pyroarsenate method was selected in preference to the usual volumetric estimation (iodine or uranium titrations) in view of the fact that any remaining organic matter might render the results unreliable[1].

From the analytical results, the ratio of arsenate to total arsenic was calculated; and from these figures, those given in the table were computed. By this means, any error due to concentration of the solutions during the passage of air was avoided. The results given are, in every case, the mean of duplicate experiments. *All the figures are expressed as* $As_2O_3$, the $As_2O_5$ being calculated as $As_2O_3$ in order to render the results more easily comparable.

As will be seen, Brünnich's results as to oxidation of arsenites to arsenates are confirmed, the reaction having taken place in the presence of wood tar and also of cresylic acids. The oxidation, however, in presence of wood tar is considerably greater than that which occurs in the case of cresylic acids. This result is of great importance, in view of the fact that Stockholm tar forms a large proportion of the Queensland Government Official Dip, which Brünnich claims to be superior to any other mixture.

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[1] Since the completion of this work, Brünnich and Smith have published a communication on the estimation of arsenates in the presence of arsenites by means of magnesia mixture (*Ztsch. f. Anorg. Chemie*, Vol. 68, p. 292). In this, it is shown that the method is only reliable when the concentration of arsenite, ammonia, etc. lies between certain limits. Methods for the estimation of arsenates in the presence of arsenites as usually given in text books are unreliable. This became obvious to us in the course of our work, and researches were undertaken to devise a suitable method. The procedure adopted above seemed to us to be the best at the time; and, as only comparative, and not absolute, results are necessary the general conclusions are not vitiated. See also Lütz and Swinne, *Ztsch. f. Anorg. Chemie*, Vol. 64, p. 298.

In Experiments IV and V, which were conducted with the solutions exposed to direct sunlight, the extent of oxidation is very much greater

| Composition (per litre) | Before passage of air | | | After passage of air | | | Difference in %  of As. present in the form of $As_2O_5$ |
|---|---|---|---|---|---|---|---|
| | Total As. per litre gms. | $As_2O_5$ per litre gms. | %  of As. present in the form of $As_2O_5$ | Total As. per litre gms. | $As_2O_5$ per litre gms. | %  of As. present in the form of $As_2O_5$ | |
| **I.**<br>5 g. *Wood tar*<br>5 g. Castor oil soap<br>10 g. $As_2O_3$<br>? $Na_2CO_3$ | 10 | 0·135 | 1·35 % | 10 | 0·789 | 7·89 % | 6·54 % |
| **II.**<br>5 g. *Wood tar*<br>5 g. Castor oil soap<br>10 g. $As_2O_3$<br>? $Na_2CO_3$ | 10 | 0·125 | 1·25 % | 10 | 0·812 | 8·12 % | 6·87 % |
| **III.**<br>5 g. *Cresylic acids*<br>5 g. Castor oil soap<br>10 g. $As_2O_3$<br>? $Na_2CO_3$ | 10 | 0·043 | 0·43 % | 10 | 0·238 | 2·37 % | 1·94 % |
| **IV.**<br>10 g. *Wood tar*<br>10 g. Castor oil soap<br>10 g. $As_2O_3$<br>10 g. $Na_2CO_3$ | 10 | 0·368 | 3·68 % | 10 | 3·289 | 32·89 % | 29·21 % |
| **V.**<br>10 g. *Cresylic acids*<br>10 g. Resin soap<br>10 g. $As_2O_3$<br>10 g. $Na_2CO_3$ | 10 | 0·656 | 6·56 % | 10 | 2·613 | 26·13 % | 19·57 % |

than in Experiments I, II and III. Doubtless in hot countries, the oxidation due to the high actinic power of the sunlight would be even greater; and it is in such countries that regular dipping of cattle is so

essential. However, until definite analytical figures, relating to freshly filled tanks and also to those which have been in use for varying periods, can be obtained, the extent of oxidation in a dipping tank can only be surmised: but these experiments confirm Brünnich's work and emphasise its importance; though whether the increased oxidation in Experiments IV and V is due to the increased amount of tar products, or to the more actinic power of the sunlight, or to a greater volume of air having been drawn through the solution, it is difficult to say.

It is a matter of experience that the concentration of arsenious oxide in a bath must be above a certain limit in order to be effective. In Queensland[1] a standard has been fixed by the Government, viz. 1 part (by weight) of arsenious oxide in 500 parts of water, or 1 lb. of arsenious oxide in 50 gallons. If 10 % of the arsenite were oxidized to arsenate, the concentration of arsenious oxide would be reduced to 1 : 555 or 1 part in $55\frac{1}{2}$ gallons of water. This last strength is below the limit necessary for the successful destruction of ticks; for the arsenic acid has very little action, as has been proved by Brünnich. If the number of cattle passing through a bath is taken into consideration, such an amount of oxidation is quite feasible. Thus, at Gonubie Park, Cape Colony, where one of the authors has carried out experimental dipping, 700 to 800 cattle were dipped per week, and this number is by no means unusual in countries where cattle raising forms one of the principal industries. Each animal makes a great splash as it enters the bath, so that the wash is effectually stirred and exposed to the atmosphere.

The Queensland Department of Agriculture not only regulates the strength of arsenious oxide in dipping baths, but also insists upon periodic analysis of their contents. On the other hand, in many countries where systematic dipping is compulsory, there are no regulations as to the maintenance of the baths in an efficient state.

At the same time, in the adoption of a standard content of arsenious oxide, it must be remembered that the actual proportion of arsenious oxide is not the only criterion; since the other constituents of a wash, have great effect on the efficiency of the arsenic. For example, a bath of pure sodium arsenite in water, containing 1 part of $As_2O_3$ to 500 of water is no more efficient than one containing 1 part of $As_2O_3$ in 600 parts of water, if a suitable emulsion of oil and soap, or oil and glue, is present.

Watkins Pitchford[2] (*Dipping and Tick-Destroying Agents*, Schedule

[1] *Queensland Ann. Rept., Dept. Agric.* 1907–8, p. 71.

[2] *Natal Agric. Journ.*, Vol. 11, p. 1576; Vol. 12, p. 436. Reprinted as Bull. 17.

11) states "Comparison...will show the result of combining other agents with this arsenite of soda, which results in the reduction of its irritating properties without taking away from its efficacy as a tick-killer."

His bath for Schedule 11 was 1 lb. of sodium arsenite to 20 gallons of water[1]. His "Laboratory Dip" contained 1 lb. of arsenite to 47 gallons. The latter, containing an emulsion, is about as effective as the former, which contains no emulsion; but it has none of the irritating properties.

The results embodied in this paper emphasise the need for official periodic analysis in all countries if dipping is to be efficient. Neglect of this precaution would result in a sense of false security and to a disbelief in the efficacy of dipping as a means of tick eradication.

[1] For the information of those persons who may be interested in comparing the content of arsenic oxide in a bath, it may be observed that the "Government Sodium Arsenite" prior to Watkins Pitchford's paper, contained 68 °/₀ $As_2O_3$. Since the date of this paper, however, an arsenite is used containing 80°/₀ $As_2O_3$. As a matter of fact, a great objection to the use of "Government Sodium Arsenite" used to be its inconstant composition (Dixon, R. W., *Agric. Journ. of Union of South Africa*, Vol. II. p. 15).

# SOIL SURVEYS AND SOIL ANALYSES.

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In undertaking a survey of the soils of a given district the methods of analysis and the classification to be adopted (which in its turn shapes the scheme of the work) must be determined by the purpose before the investigator at the outset. His end may be scientific—to assign the soils in question to their types, or practical—to give information to the cultivator by showing him the area over which a given method of treatment may be expected to succeed. Much will depend upon the extent of the area under investigation and on the character of the climate prevailing. For example the genetic classification of soils such as has been suggested by Sibirtzef (see this *Journal*, 1909, III. 80) divides soils into a series of great types which are really determined by climatic zones. In the steppe area, for example, where the black soils (Tchernozem) prevail, climatic conditions have led to the accumulation of large proportions of mild or neutral humus until it has become the dominant factor in determining the character of the soil; whether the original substratum be sand or clay the amount of organic matter causes the soil to work lightly and yet retain moisture. In such an area, and the area will in the nature of things be large, soil analyses will be of little value because all differences, chemical or physical, in the nature of the substratum will be over-ridden by the preponderance of the humus. Such an area again will show little relation between the soil and the geology of the country, the soil being practically a drift deposit which has overgrown all the underlying formations alike. In the United Kingdom we must regard ourselves as living almost entirely within one only of these large climatic zones but one which does not superimpose a soil type on all the various strata to be found there, so that a great diversity of soils may occur within a very small area. We do see the climatic divisions in the peaty soils which develop in all parts of

the country above a certain elevation, for though some differences may be traced in the vegetation carried by the moorlands, in the main the character of the soils is alike and has been determined by the elevation and the rainfall and not by the nature of the underlying formation. As a rule however in the United Kingdom the soil is derived from and shares the character of the rock or drift material below; even where there might have once been a common forest or steppe soil over a considerable area the processes of cultivation carried on for so long have obliterated the excess of organic matter and given the underlying differences full play. The character of our climatic zone is such as to accentuate variety of soils, the humidity is considerable and carries the degradation of the rock material so far that there is an enormous range in the sizes of the particles making up the soil, from coarse sand grains down to clay particles of the order of colloids; there has also been sufficient accumulation of humus to modify the texture of the mass and make it work as a unit and not as a loose aggregate of powdered rock. Chemically also our soils lie between the semi-arid soils with their richness in unleached salts derived from the decayed rock, and the "podzols" from which all soluble material has been washed away. Other circumstances call for soil surveys of a detailed character with some minuteness of analysis. Over a great part of the country and particularly in the highly farmed districts of England a considerable number of formations are represented, sharply contrasted in their nature and dipping at a deep angle so that the soil changes radically within comparatively small distances, and a farmer of no more than two or three hundred acres may find two or three distinct types of soil upon his own land. Further, with the intensive farming that is practised here and the long tradition that exists as to the behaviour under cultivation of every field, in our farming it is possible and indeed necessary to secure a much closer correlation of crop and manure to the soil, and of methods of cultivation to the soil and weather, than is required where the object is a cheaply grown product over a wide area even if the yield per acre is small. Thus the differentiation of soil types and their characterisation must be pushed as far as experience shows recognisable variations in practice; it will not be sufficient to class together as clays soils derived from the London Clay and the Weald Clay, when farmers upon the one area find that they need to work their soil somewhat differently from those situated upon the other, though many classifications and even methods of analysis would regard the two soils as alike.

The following discussion is based upon a survey of the soils of the south-east of England, which has been recently published by the Board of Agriculture[1] and to which reference must be made for the general details as to the district and its agriculture that are needless here. It must however be pointed out that this is a very special land surface made up of the outcrop of no less than 18 formations running in narrow U-shaped strips round the area, which formations themselves also show certain definite changes in lithological character in different parts of the area. Widespread drift is also absent so that in most cases the soils are truly sedentary or autochthonous ; even the big areas of drift, such as the " Clay-with-Flints " or the " Brick Earths," are uniform enough to be treated as formations giving rise to their own types of soil. There is nothing in this area to correspond to the great stretches of Glacial Drift prevailing in more northern parts of the country, where a formation which the geologist can only map under a single name may vary within a short distance and with no apparent regularity from the coarsest sand to heavy clay. Climate does not vary greatly within the area, though, as will be shown, a difference in the rainfall exists sufficient to affect some of the farming practices. The conclusions we have been led to as to the correlation of certain crops and modes of farming with soils of given composition, can therefore only be regarded as holding within the limits of climate prevailing in the area under investigation.

The south-east of England forms a well-defined geological unit; it is an anticlinal dome of all the strata between the Purbeck and the Eocene, the fold has been denuded down to the Lower Wealden core and then valleys have been carved out by aerial denudation that run parallel to the fold from east to west, these valleys representing the softer strata, of which the edges were laid bare on the original paring down of the fold. The western end of the dome is complete, but the eastern extremity is cut across by the straits of Dover, so that the formations show a series of parallel U-shaped outcrops encircling a central core. This centre—the High Weald—is an elevated mass of Lower Wealden sands and clays surrounded by a broad low plain of Weald Clay ; round this comes the Lower Greensand series, presenting as a rule a steep scarped face towards the core. This in its turn is succeeded by another valley formed by the Gault Clay, which is everywhere surrounded by the high Chalk escarpment. On the back or

[1] A. D. Hall and E. J. Russell, "A Report on the Agriculture and Soils of Kent, Surrey, and Sussex," *Board of Agriculture and Fisheries*, 1911.

dip-slope of the chalk thin beds of Eocene strata occur, but with some irregularity, so that they do not give rise to the continuous valleys of the lower formations. From east to west the area is roughly 100 miles long and about 50 miles from north to south.

The objects we had before us may be summed up as follows :

(1) To show the distribution of soils of similar agricultural properties, and to define these soils by some method of analysis.

(2) To trace such correlations as exist between the chemical and physical properties of the soils and the crops and agricultural methods that are actually associated with them.

(3) On the basis of the observed distribution of the soil types and the ascertained associations to afford guidance as to cropping and manuring over the whole area.

It will be thus seen that the basis of the work was essentially empirical ; the agricultural properties of the soil type were ascertained by observation, the distribution of the crops was determined by constructing maps from the parish returns of the acreage under each crop, and the rotations followed and special methods of cultivation adopted in the various areas were learnt by personal enquiry. We are not as yet in a position to deduce the agricultural properties of a soil, either its behaviour under cultivation or its adaptability to particular crops, from its analysis, except in the roughest general fashion ; but by the accumulation of analytical data about soils of known behaviour we may be able to work out their characteristic composition and even to explain the cause of their special properties. For example, we cannot draw up, from first principles, the specification of a soil good for fruit growing, but by correlating the analyses of a large number of soils which have in practice proved suitable for fruit we may find certain features in common, which in future may be taken as determining the nature of a fruit soil. Even on the chemical side the same thing is true; the significance of a given amount, for example 0·1 per cent. of phosphoric acid in a soil of a given type, cannot be laid down *a priori*, it must be ascertained from the results of field experiments upon the area in question. At its best soil analysis only reveals one set of factors in plant production, and these have to be interpreted in the light of local conditions, such as climate, water-supply and drainage.

As it would be almost out of the question to construct a soil map on a basis of analysis only, examining for example field by field along the common boundary of two types of soil in order to draw that boundary, some guiding principle must be sought for, and this in the

area in question has been provided by the Geological Survey. It was a matter of experience that within the district there was a general correlation between soils and geological outcrop, and at the outset a number of determinations were made to ascertain if the outcrop lines laid down on the geological map would also serve as boundary lines between two soil types.

Of course the 'drift' maps of the Geological Survey were employed; in the area examined two widespread drift formations occur, the Clay-with-Flints and the Brick Earth, which give rise to soils quite distinct from those originating from the Chalk by which they are usually underlaid. Only a map on the scale of one mile to the inch was available, but as the work progressed and both analyses and information as to the agriculture accumulated, our initial conclusion was confirmed and it became certain that each geological outcrop did, with certain provisos to be considered later, give rise to its own special soil type. Boundaries between neighbouring soils could be drawn, except in certain cases, with considerable accuracy, and were coincident with the boundaries laid down on the geological map. Adopting this geological system of classification a number of samples were then taken along the outcrop of each formation, and when the formation was broad traverses were also made across its breadth to ascertain if sensible differences in the soil were induced by the higher and lower beds of the formation.

In drawing the samples fields were selected that had not been much disturbed by small local rearrangements of the material, *i.e.* steep slopes and hollows, stream bottoms and any places that indicated made ground, were avoided. A composite sample made up of six or eight cores bored out with a cylindrical auger two inches in diameter was taken to represent each field, the bores being scattered about the field well away from the hedges, gateways and buildings. Unless some sharp change indicated that a less depth was necessary the top nine inches was regarded as soil, the second nine inch layer as subsoil.

Inspection of the results showed at once that only the mechanical analyses possessed any general consistency or could characterise the formation in any way; the mechanical analyses did however indicate a distinct soil type for each formation. As might however be expected in an area of such narrow outcrops and steep slopes the range of variation between samples taken a few miles, or even a few fields, apart was much greater than the errors of analysis; but though the numbers of analyses are far from sufficient for adequate discussion, it is now possible

to draw some conclusions as to the degree of uniformity to be expected and the value of the resulting soil type.

The following cases occur:

(1) The formation is uniform within comparatively small limits of variation. A good example is afforded by the Folkestone Sands, the highest bed of the Lower Greensand series which gives rises to barren

TABLE I.*

*Folkestone beds.*

| | East Kent | | Surrey | | West Sussex |
|---|---|---|---|---|---|
| | 78 | 13 | 124 | 30 | 192 |
| Fine gravel, above 1 mm.... | 0·9 | 0·9 | 2·5 | 2·8 | 0·8 |
| Coarse sand, 1—0·2 mm. ... | 54·3 | 63·3 | 52·6 | 56·7 | 59·7 |
| Fine sand, 0·2—0·04 mm.... | 16·7 | 18·0 | 26·2 | 24·7 | 22·1 |
| Silt, 0·04—0·01 mm......... | 8·1 | 5·0 | 4·8 | 2·9 | 3·9 |
| Fine silt, 0·01—0·002 mm.... | 5·6 | 4·0 | 3·5 | 2·4 | 3·8 |
| Clay, below 0·002 mm. ...... | 5·7 | 3·1 | 3·8 | 0·8 | 2·7 |
| *Subsoil* | | | | | |
| Fine gravel, above 1 mm.... | 0·3 | — | 3·0 | ... | 1·6 |
| Coarse sand, 1—0·2 mm. ... | 57·2 | -- | 55·7 | — | 66·7 |
| Fine sand, 0·2—0·04 mm.... | 16·9 | — | 23·5 | ... | 20·9 |
| Silt, 0·4—0·01 mm. ......... | 9·1 | – | 3·8 | —- | 2·9 |
| Fine silt, 0·01—0·002 mm.... | 4·5 | ... | 1·9 | | 3·9 |
| Clay, below 0·002 mm. ...... | 5·7 | — | 4·5 | — | 1·5 |

\* The numbers by which the soils are designated here are the same as those used in the Soil Report already referred to.

heaths all along its outcrop. On examining the analyses set out in Table 1 one fact at once stands out, the great preponderance among the soil fractions of the "coarse sand," 1—0·2 mm., which constitutes 55—65 per cent. of the whole soil. The "fine sand" is the next most abundant fraction, 18—25 per cent., while the clay or fine silt together only amount to 3—7 per cent.

In the district there are three other sandy formations giving rise to equally barren heaths with the same type of · vegetation— the Hythe Beds of the Lower Greensand in the west of Surrey, the Bagshot Beds, and the Woolwich Beds of the Lower Eocene to the south-east of London—yet the soils of these formations are quite distinct in that they never contain the same proportion of coarse sand. At the most the coarse sand constitutes 30 per cent., the predominant

fraction being the fine sand which runs up to 66 per cent., while the finer fractions of clay and fine silt amount to much the same in all the soils. Thus all the soils derived from the Folkestone Beds are clearly distinguished from other sandy soils and they preserve their character over a considerable area.

Another case in point is afforded by the very fertile Brick Earths, which are considerably developed as a shelf of probably Post Pliocene alluvial origin overlying the Chalk and Eocene along the seaward side of East Kent.

TABLE II.

*Brick Earths, East Kent.*

|  | 112 | 120 | 129 | 100 | 133 |
|---|---|---|---|---|---|
| *Soil* | | | | | |
| Fine gravel, above 1 mm. ...... | 0·8 | 0·3 | 0·3 | 1·0 | 0·7 |
| Coarse sand, 1—0·2 mm. ...... | 1·2 | 0·8 | 0·7 | 3·0 | 2·1 |
| Fine sand, 0·2—0·04 mm. ...... | 27·5 | 30·2 | 24·7 | 27·2 | 39·1 |
| Silt, 0·04—0·01 mm. ........... | 40·9 | 43·7 | 44·8 | 40·0 | 26·1 |
| Fine silt, 0·01—0·002 mm....... | 9·8 | 7·8 | 8·6 | 8·9 | 8·6 |
| Clay, below 0·002 mm. ......... | 13·1 | 10·4 | 14·7 | 11·2 | 11·7 |
| *Subsoil* | | | | | |
| Fine gravel, above 1 mm. ...... | 0·2 | 0·3 | Nil | 0·2 | 0·5 |
| Coarse sand, 1—0·2 mm. ...... | 0·7 | 0·4 | 0·3 | 1·9 | 1·3 |
| Fine sand, 0·2—0·04 mm. ...... | 26·4 | 29·5 | 25·3 | 25·3 | 41·7 |
| Silt, 0·04—0·01 mm. ........... | 38·1 | 37·8 | 39·5 | 41·4 | 25·3 |
| Fine silt, 0·01—0·002 mm....... | 10·8 | 10·7 | 9·0 | 9·6 | 8·6 |
| Clay, below 0·002 mm. ......... | 15·2 | 14·6 | 12·2 | 14·5 | 14·3 |

In this material there are very few stones, and, as Table II shows, but little material of more than the 0·2 mm. grade, while soil and subsoil are very similar in character. The " fine sand " varies between 25 and 30 per cent., the silt constitutes about 40 per cent. (in one sample, No. 133, these proportions are reversed but the total is the same), while fine silt only amounts to 8 per cent. and clay to 12 per cent.

The chemical composition of these soils is very similar, they are all rich in organic matter for arable soils, containing 5—6 per cent. in the surface and 3 per cent. in the subsoil, the calcium carbonate is constant at about 0·1 per cent. except where there has been artificial additions of chalk, total potash is about 0·4 per cent. and phosphoric acid about 0·1 per cent. Local as is the distribution of this drift stratum the conditions under which it was deposited must have been very uniform to give rise to so regular a product.

(2) Uniformity of this high order is however the exception in our area, more often a gradual change sets in either in passing along the outcrop or in ascending it from the lower to the higher beds. This may be attributed to the fact that many of the formations dealt with, the Wealden Beds and the Lower Greensand, were laid down in estuaries of no very great size with the usual result that the coarser particles were deposited close in shore while the finer particles only settled further out where the currents were more gentle.

The most striking change of this kind is shown by the member of the Lower Greensand known as the Hythe Beds. At its eastern extremity in Kent it forms an argillaceous limestone giving rise to

TABLE III.

*Hythe Beds.*

| | I. Eastern portion | | | II. Central portion | | | III. Western portion | | |
|---|---|---|---|---|---|---|---|---|---|
| | East Kent loams | | | Mid-Kent Ragstone | | | Surrey and West Sussex Sands | | |
| | 149 | 93 | 187 | 185 | 127 | 152 | 168 | 50 | 205 |
| *Soil* | | | | | | | | | |
| Fine gravel, above 1 mm.... | 0·9 | 0·8 | 1·4 | 2·2 | 2·3 | 3·5 | 4·2 | 0·1 | 2·6 |
| Coarse sand, 1—0·2 mm... | 16·8 | 19·9 | 10·6 | 7·8 | 9·5 | 10·2 | 12·7 | 12·6 | 9·5 |
| Fine sand, 0·2—0·04 mm. | 28·7 | 28·8 | 29·4 | 33·6 | 30·6 | 33·5 | 51·6 | 67·4 | 67·1 |
| Silt, 0·04—0·01 mm. ...... | 9·5 | 17·0 | 18·7 | 13·0 | 19·7 | 14·6 | 10·9 | 5·9 | 5·2 |
| Fine silt, 0·01—0·002 mm. | 9·5 | 9·0 | 14·0 | 17·8 | 11·1 | 14·9 | 5·5 | 5·2 | 6·6 |
| Clay, below 0·002 mm....... | 18·8 | 14·6 | 13·7 | 15·0 | 13·3 | 12·2 | 3·4 | 2·2 | 5·0 |
| *Subsoil* | | | | | | | | | |
| Fine gravel, above 1 mm.... | 0·8 | 0·3 | 1·6 | 1·8 | 2·2 | 2·6 | 4·8 | — | 1·1 |
| Coarse sand, 1—0·2 mm.... | 17·2 | 18·1 | 12·7 | 6·0 | 11·5 | 9·8 | 6·0 | — | 9·6 |
| Fine sand, 0·2—0·04 mm. | 30·7 | 26·6 | 29·1 | 31·8 | 28·1 | 30·2 | 58·5 | | 70·1 |
| Silt, 0·04—0·01 mm. ...... | 11·7 | 19·4 | 19·5 | 13·9 | 18·7 | 17·5 | 9·2 | .. | 6·1 |
| Fine silt, 0·01—0·002 mm. | 8·5 | 9·8 | 10·1 | 17·5 | 13·1 | 15·5 | 5·7 | . | 5·9 |
| Clay, below 0·002 mm....... | 19·9 | 16·0 | 16·3 | 19·5 | 16·2 | 15·3 | 3·2 | — | 5·4 |

fairly fertile soils of medium texture, very distinct from the Folkestone sands above. In Mid-Kent the rock is still a calcareous sandstone, but the resulting soils are lighter and form an area of great fertility, " the Ragstone," specially prized for the growth of fruit and hops and other valuable crops. A few miles further west the carbonate of lime disappears from the rock, which becomes exclusively sandy, in many

places to the extent of losing all coherence; the soils in consequence become infertile and are occupied to a large extent by waste heaths and pine woods all through Surrey and round the southern outcrop in West Sussex.

Over this western area the soils from the Hythe Beds are almost indistinguishable except on analysis from those derived from the adjacent Folkestone Beds, though the greater coarseness of the latter is still reflected in the larger proportion of waste. Though soils derived from this formation carry barren heaths on the one hand and some of the most fertile fruit and hop grounds on the other, it can for all practical purposes be marked off into three areas—the extreme eastern rather heavy loams, the light "Ragstone" loams in Mid-Kent, and the pure sands from Sevenoaks westwards. Within these areas the soils are similar, and though one area grades off into another with no distinct dividing line, it is not difficult to forecast the type of soil likely to be found in any part even of the debatable land on the boundaries.

The London Clay affords another example; in Kent and especially in East Kent it gives rise to extremely heavy soils containing as much as 40 per cent. of clay; moving eastward it gradually lightens in character and, though still a heavy clay to the south of London, in West Surrey it has become little more than a clay loam fit for arable cultivation, containing some 15 per cent. of coarse sand, 30 to 40 per cent. of fine sand (both of which fractions are practically absent from the Kent samples) and only 15 to 20 per cent. of clay.

Lastly we may instance the Weald Clay. This formation gives rise to soils that are quite distinct from those derived from the London Clay in that the "fine silt" fraction is as large or even larger than the "clay" fraction, while the coarse sand fraction is very small indeed. These soils again are heaviest to the east and become mixed with a certain amount of sand in West Surrey, where also much of the Weald Clay is under arable cultivation. The analyses in Table IV are arranged geographically from east to west, and the progressive change in composition is evident.

(3) A third case is provided by those formations which give rise to a series of soils varying considerably within short distances and on no very obvious system, but yet preserve a certain unmistakable similarity of type. This is very well seen in the soils derived from the Lower Wealden formation, which consists of alternations of sand and clay that can to some degree be geologically differentiated though the outcrops are local and variable. But though the soils thus change

from very stiff to light sands, there is a marked general resemblance running through all of them; they all work more heavily than would be expected, turning up very solid under the plough and never

## TABLE IV.

### *London Clay.*

|  | I. Eastern portion | | II. Central portion | | III. Western portion |
|---|---|---|---|---|---|
|  | 65 | 67 | 294 | 107 | 26 |
| *Soil* | | | | | |
| Fine gravel, above 1 mm. ... | 0·4 | 0·5 | 1·7 | 0·4 | 0·6 |
| Coarse sand, 1—0·2 mm. . | 0·8 | 0·3 | 18·4 | 12·8 | 17·4 |
| Fine sand, 0·2—0·04 mm.... | 6·5 | 17·6 | 12·7 | 25·5 | 38·1 |
| Silt, 0·04—0·01 mm. ... ..... | 15·8 | 13·4 | 16·6 | 11·3 | 14·0 |
| Fine silt, 0·01-0·002 mm.... | 16·3 | 15·3 | 11·1 | 11·1 | 7·3 |
| Clay, below 0·002 mm. ...... | 40·5 | 36·8 | 24·6 | 23·7 | 12·8 |
| *Subsoil* | | | | | |
| Fine gravel, above 1 mm.... | 0·1 | Nil | 1·3 | 0·5 | 0·2 |
| Coarse sand, 1—0·2 mm... | 0·5 | 0·1 | 23·6 | 6·7 | 13·7 |
| Fine sand, 0·2—0·04 mm.... | 4·6 | 14·4 | 11·3 | 15·4 | 29·8 |
| Silt, 0·04—0·01 mm......... | 9·3 | 10·8 | 18·0 | 11·9 | 15·0 |
| Fine silt, 0·01—0·002 mm.... | 19·3 | 16·5 | 11·4 | 12·9 | 9·5 |
| Clay, below 0·002 mm...... | 47·8 | 42·0 | 24·9 | 36·4 | 20·8 |

### *Weald Clay.*

|  | I. Eastern portion | | | II. Central portion | | III. Western portion | | |
|---|---|---|---|---|---|---|---|---|
|  | 70 | 287 | 692 | 86 | 74 | 51 | 215 | 196 |
| *Soil* | | | | | | | | |
| Fine gravel, above 1 mm. | 0·9 | 0·8 | 1·3 | 0·1 | 1·0 | 12·4 | 1·8 | 0·9 |
| Coarse sand, 1—0·2 mm. | 1·1 | 1·5 | 2·1 | 2·4 | 1·9 | | 6·6 | 11·4 |
| Fine sand, 0·2—0·04 mm. | 9·3 | 8·7 | 19·4 | 12·3 | 10·8 | 28·5 | 33·8 | 43·2 |
| Silt, 0·04—0·01 mm. ...... | 25·9 | 13·7 | 20·5 | 23·2 | 20·7 | 18·5 | 20·3 | 13·0 |
| Fine silt, 0·01—0·002 mm. | 24·4 | 31·6 | 25·9 | 23·8 | 28·6 | 15·7 | 8·2 | 10·2 |
| Clay, below 0·002 mm. ... | 28·6 | 27·8 | 19·4 | 23·9 | 20·7 | 10·7 | 13·0 | 10·9 |
| *Subsoil* | | | | | | | | |
| Fine gravel, above 1 mm. | 0·7 | — | — | 1·3 | 4·7 | .... | 1·6 | 0·6 |
| Coarse sand, 1—0·2 mm. | 1·1 | —— | — | 4·6 | | .... | 6·3 | 21·4 |
| Fine sand, 0·2—0·04 mm. | 9·0 | — | —— | 25·6 | 11·0 | —— | 27·7 | 34·2 |
| Silt, 0·04—0·01 mm. ...... | 18·8 | — | — | 32·1 | 19·6 | —— | 23·2 | 10·6 |
| Fine silt, 0·01—0·002 mm. | 26·5 | . .. | — | 14·6 | 28·3 | . | 14·2 | 10·4 |
| Clay, below 0·002 mm. ... | 37·8 | —— | .... | 15·4 | 23·3 | — | 18·7 | 14·0 |

*Soil Surveys and Soil Analyses*

crumbling readily even with good cultivation. They run freely in heavy rain and then dry with a tenacious crust on the surface and a characteristic white aspect which often leads strangers to regard the soil as chalky. The subsoil always shows a mottled yellow-brown appearance at a depth of a foot or so and often contains small concretions of clay iron-stone. An examination of the mechanical analyses of these Lower Wealden soils shows one striking feature in common— the almost total absence of the coarser fractions like fine gravel or coarse sand, together with an exceptional amount of silt and fine silt.

TABLE V.

*Lower Wealden Beds.*

| | Ashdown beds | | | T. Wells beds | | | Wadhurst clay | | |
|---|---|---|---|---|---|---|---|---|---|
| | 197 | 241 | 244 | 246 | 242 | 289 | 239 | 172 | 179 |
| *Soil* | | | | | | | | | |
| Fine gravel, above 1 mm. | 0·8 | 0·2 | 3·5 | 1·6 | 1·1 | 1·8 | 1·1 | 2·9 | 0·4 |
| Coarse sand, 1--0·2 mm. | 0·3 | 0·3 | 0·8 | 5·0 | 0·8 | 3·3 | 0·4 | 1·8 | 0·5 |
| Fine sand, 0·2—0·04 mm. | 35·2 | 53·2 | 25·3 | 27·4 | 47·3 | 36·8 | 35·9 | 13·2 | 24·7 |
| Silt, 0·04—0·01 mm. ...... | 35·2 | 19·9 | 27·1 | 33·8 | 18·6 | 20·9 | 21·6 | 20·4 | 30·1 |
| Fine silt, 0·01—0·002 mm. | 15·8 | 10·1 | 21·5 | 14·3 | 12·4 | 14·3 | 15·0 | 22·4 | 19·7 |
| Clay, below 0·002 mm. ... | 5·4 | 5·9 | 12·5 | 9·7 | 12·4 | 13·5 | 15·7 | 25·1 | 14·9 |
| *Subsoil* | | | | | | | | | |
| Fine gravel, above 1 mm. | ... | — | 2·0 | 3·9 | 2·0 | 2·5 | 0·2 | 2·9 | 0·3 |
| Coarse sand, 1—0·2 mm.... | — | — | 0·9 | 4·7 | 0·4 | 2·8 | 0·0 | 1·2 | 0·1 |
| Fine sand, 0·2—0·04 mm. | — | — | 23·9 | 23·2 | 46·2 | 35·2 | 39·4 | 11·8 | 18·0 |
| Silt, 0·04—0·01 mm. ...... | — | — | 27·1 | 34·0 | 16·3 | 19·9 | 19·7 | 26·8 | 34·3 |
| Fine silt, 0·01—0·002 mm. | — | — | 20·6 | 15·9 | 10·6 | 16·1 | 12·5 | 16·2 | 22·3 |
| Clay, below 0·002 mm. ... | — | — | 17·3 | 11·1 | 16·6 | 16·1 | 21·2 | 22·8 | 17·0 |

Even the sandiest of the soils contains only fine sand with a considerable proportion of silt, while the heavy soils are not especially rich in clay and often show a larger fine silt fraction. The soils are in fact composed in the main of sand, always fine even in the confessedly sandy soils and running down to a fine silt in the heavier examples.

Detailed chemical analyses of the clay fraction (see p. 199) show that the clay is rather different in character from that occurring in the more fertile soils: as a rule the percentage of alumina in the soil extracted by hydrochloric acid is approximately one-third of the clay determined by the mechanical analysis, but in the Lower Wealden soils it is invariably below this proportion, constituting on the average only about 15 per cent. of the clay. Similarly the

potash extracted by hydrochloric acid is only about 1 per cent. of the clay, instead of 3 per cent. as is usually the case. The agricultural properties of these soils also indicate that the clay fraction differs from that of more fertile soils in that it cannot readily be flocculated by the weather or by chemical means; there is thus great difficulty in securing a fine crumbly tilth. The white aspect of the soils when dry can be referred on close examination to the way the very fine white sand is left on the surface of the clods after a little rain, it is another sign of the ease with which these unflocculated soils will wash. On some of the Rothamsted plots where flocculation of the clay has been destroyed by chemical means the soil similarly dries with a white look due to the skin of fine sand particles left on the surface of the clods. The tenacity of the plough slice may be set down to the lack of coarse sand.

We must regard these Lower Wealden soils as constituting a single type, possessing common characteristics, though the individual soils making up the group show very varying degrees of heaviness and lightness; within its own limits this type possesses soils approximating to sands and clays, but both are peculiar and unlike normal sands and clays.

## On taking the Sample of Soil.

We have in the preceding discussion assumed that a sample of soil can be taken from a particular spot representative of the soil for some distance around it; unless this is so the analysis is obviously useless. If a report is wanted on a particular field it is not difficult to take samples at a number of places fairly close to one another and to draw from these an average sample on which the determinations may be made. But in dealing with a geological formation for the purposes of a soil survey such close sampling is clearly impossible, and we have now to find how far any considerable stretch of country can be represented by one or two samples.

It is evident that the stretch of soil must be tolerably uniform. An essential preliminary to a soil survey is therefore a careful inspection of the whole of the formation with a view to its division into areas within which the agricultural conditions are fairly uniform; for this purpose the character of the soil and the vegetation, the type of farming and the value of the land all afford useful indications. Within each area a number of points must then be chosen at which samples are to be taken. These points must be distributed at not too great

a distance and must represent the area as well as possible; *e.g.* they should not be taken on steep slopes unless these are the chief feature of the area, but rather on level patches, on long gentle slopes, or in large valleys where the risk of admixture with other formations is reduced to a minimum. The object of the survey being to correlate the composition of the soil with its agricultural properties very full enquiries should be made from the farmer as to the agricultural value of the land, the crops and manures most suitable, the behaviour of the soil during drought and wet weather, and any special points to be observed during cultivation. Information is also wanted about the most troublesome weeds, the native vegetation, hedgerow and other timber, etc.

The methods of analysis must show the fundamental properties of the soil and not the transient properties conferred upon it by agricultural treatment. If for example, there are three fields of identical origin the methods must bring out this uniformity, though one field has long been down in grass, another has been highly farmed, and the third has been worked out. The analysis thus gives information about the inherent capabilities of the soil; on this basis alone can any comparison and classification be based. Once the fundamental similarity of two soils is established it is not a difficult matter to find out in what chemical or physical respects they differ, if any striking agricultural difference is manifest between them. Soil analysis as yet can only compare soils; it can say that two are really alike and that results achieved on the one might be achieved on the other if certain methods were adopted, but it cannot go further. It is important that this limitation should be borne in mind, and that soils selected for examination within each of the areas should be carefully chosen so that they may serve as standard soils when further enquiries are sent in.

In our experience mechanical analysis gives by far the most useful information in comparing soils. The following table shows the kind of agreement obtained when soils from the same field, or from fields not far apart, are analysed.

The variation is usually about 5 to 10 per cent. from the mean; these limits are found to hold in other cases over similar areas, and may be taken as representing the minimum variation. It will be seen also that the method fulfils the essential condition that it should not be obscured by the agricultural treatment of the land; both at Rothamsted and at Nutfield land under arable cultivation and grass show their fundamental similarity in spite of their difference in treatment for thirty

## TABLE VI.

*Mechanical analysis of samples of soil taken over a small area.*

| Formation | London clay | | | Clay-with-flints | | | Folkestone beds | | Hythe beds | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality | Merton, Surrey | | | Rothamsted | | | Nutfield, Surrey | | Near Midhurst, Sussex | | |
| | Different parts of the same field | | | Different parts of the same field | | | Two fields 1 mile apart | | Woodland | Arable | |
| | Bottom of field | Middle of field | Top of field | Pasture, 30 yrs. old | Arable portion 1 | Arable portion 2 | Grass, 30 yrs. old | Arable | | 1 mile away | 3 miles away |
| | 290 | 291 | 292 | | 1 | 2 | 101 | 102 | 204 | 205 | 248 |
| **Soil** | | | | | | | | | | | |
| Fine gravel, above 1 mm. | 0·6 | 0·5 | 0·2 | 1·7 | 3·5 | 3·2 | 2·3 | 2·9 | 1·4 | 2·6 | 1·4 |
| Coarse sand, 1—0·2 mm. | 24·0 | 25·8 | 23·6 | 1·4 | 5·8 | 4·6 | 42·9 | 46·6 | 10·1 | 9·5 | 9·3 |
| Fine sand, 0·2—0·04 mm. | 15·2 | 17·5 | 11·7 | 16·6 | 18·8 | 20·5 | 27·4 | 22·9 | 68·8 | 67·1 | 68·5 |
| Silt, 0·04—0·01 mm. | 17·9 | 15·5 | 17·6 | 27·5 | 21·7 | 25·7 | 6·6 | 3·5 | 4·7 | 5·2 | 3·6 |
| Fine silt, 0·01—0·002 mm. | 11·8 | 11·0 | 9·4 | 13·2 | 11·0 | 11·3 | 5·8 | 8·8 | 5·7 | 6·6 | 5·6 |
| Clay, below 0·002 mm. | 22·0 | 21·5 | 25·7 | 22·0 | 21·6 | 23·7 | 7·7 | 6·9 | 3·6 | 5·0 | 5·5 |
| **Subsoil** | | | | | | | | | | | |
| Fine gravel, above 1 mm. | 0·2 | 0·3 | 0·3 | — | — | — | 2·1 | • | — | 1·1 | 0·4 |
| Coarse sand, 1—0·2 mm. | 23·5 | 22·0 | 16·6 | — | — | — | 44·9 | — | — | 9·6 | 10·0 |
| Fine sand, 0·2—0·04 mm. | 11·4 | 16·2 | 9·1 | — | — | — | 21·9 | — | — | 70·1 | 70·4 |
| Silt, 0·04—0·01 mm. | 17·2 | 16·5 | 15·8 | — | — | — | 5·2 | — | — | 6·1 | 3·5 |
| Fine silt, 0·01—0·002 mm. | 8·4 | 8·5 | 9·3 | — | — | — | 8·4 | — | — | 5·9 | 5·2 |
| Clay, below 0·002 mm. | 28·6 | 27·5 | 37·7 | — | — | — | 11·1 | — | — | 5·4 | 6·1 |

years.  This desirable result is only attained, however, when the soil is
treated with dilute acid previous to the mechanical analysis; an in-
vestigation made by one of us has shown that water alone without the
acid is insufficient to break up the temporary aggregates formed by
cultivation[1].

*Subsoil.*  In mechanical analysis the subsoil is not very different
from the surface except in containing more clay, particularly in the
heavier soils as shown in Table XV.  This close resemblance empha-
sises the comparative unalterability of the particles; some of the soils
have long been in cultivation and the surfaces have for immense periods
of time been exposed to weathering from which the subsoil is protected,
yet no measurable change has been produced.  In the absence of drifts
and surface washings the subsoil affords no better characterisation of
the formation than does the surface soil, but it is always desirable to
examine both; indeed in no other way can the absence of transported
material from the sample of surface soil be demonstrated.

For purposes of the mechanical analysis it is therefore immaterial
what crop is on the ground and whether the sample is taken from
grass or arable land.  For the chemical analysis it is desirable to have
representative soils of arable and of grass land and of land devoted to
any special crops as hops or fruit, if they play any large part in the
agriculture of the district.  On the whole, therefore, it is most useful
to let the samples represent the area as fully as possible ; if the farming
is diversified there should be a corresponding diversity in the samples,
if it is highly specialised the samples may be largely confined to the
particular crop that is grown.  Much information can often be obtained
by taking a few samples of waste land.

### Extent of Variation in Soils on the same Formation where the Formation is Uniform.

We have already discussed the effect of a change in the character
of the rock in passing along or across a formation.  Within the areas
constituting a single soil type there may still be variation even though
the rock remains unaltered.  A very common case arises from the
burying of the surface soil under deposits washed down from a higher
point or left by repeated floods.  Thus soils at a higher level tend to
become shallower than those lower down ; the change is obvious when
the underlying material is rock or chalk but less so where a clay soil

[1] Hall, *Trans. Chem. Soc.* 1904, **85**, 950.

lies on clay subsoil; in which case near the top the soil is so thinned down as to include part or all of what was originally the subsoil. Table VII shows examples where the subsoil of one part of the field is practically identical with its surface soil, which in turn resembles the surface soil of the higher ground. The original subsoil at Merton was identical with that found on the higher ground, the present subsoil of the lower ground having been in all probability the original surface which has since become buried. Hamsey Green and Woodchurch afford similar instances: in sample 110 we probably see the true surface and subsoil as they were formed, but in 109 the original surface soil has

TABLE VII.

*Variation in soil due to washing or flooding.*

| Formation ... | London clay | | | | Clay-with-flints | | | | Weald | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality ...... | Merton, Surrey | | | | Hamsey Green, Surrey | | | | Woodchurch, Kent | | | |
| | Lower ground 294 | | On the hill 293 | | 109 | | 110, 200 yards away | | 69 | | 70 | |
| | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil |
| Fine gravel ... | 1·7 | 1·3 | 1·5 | 0·3 | 1·6 | 3·1 | 1·7 | 1·4 | 0·5 | 0·6 | 0·9 | 0·7 |
| Coarse sand | 18·4 | 23·6 | 16·9 | 8·4 | 9·5 | 6·7 | 5·3 | 7·1 | 2·5 | 1·9 | 1·1 | 1·1 |
| Fine sand ... | 12·7 | 11·3 | 12·4 | 12·7 | 22·3 | 28·0 | 28·7 | 25·1 | 14·7 | 13·0 | 9·3 | 9·0 |
| Silt ............ | 16·6 | 18·0 | 16·6 | 13·4 | 25·4 | 22·5 | 26·3 | 17·6 | 24·2 | 27·8 | 25·9 | 18·8 |
| Fine silt ...... | 11·1 | 11·4 | 10·1 | 9·8 | 9·9 | 12·6 | 10·2 | 9·5 | 23·7 | 23·3 | 24·4 | 26·5 |
| Clay ............ | 24·6 | 24·9 | 26·7 | 41·7 | 16·0 | 16·4 | 16·4 | 28·3 | 20·1 | 28·9 | 28·6 | 37·8 |

become covered by transported material. In the Woodchurch soils we may either suppose that 70 has lost its original surface soil, the present surface being the bared subsoil, so that 69 would represent the normal state of things or that 69 has been covered with a deposit of rather lighter type whilst 70 represents the original condition.

This kind of variation is very common and shows that many of our soils must have suffered from similar changes. In consequence a fair number of samples are wanted for mechanical analysis, no matter how carefully geological considerations have been taken into account in the selection, and the lack of agreement sometimes throws a good deal of light on soil problems that appear at first obscure.

## INTERPRETATION OF THE RESULTS OF A MECHANICAL ANALYSIS.

Mechanical analysis shows at once whether two soils are similar, or if not, how they differ, but several difficulties arise when one begins to discuss the results or to attempt an interpretation of them. The fractions—except the clay and part of the fine silt—do not represent distinct substances; the lines are all artificial and merely divide up into a convenient number of groups a mixture showing a perfect gradation from an upper down to a lower limit. This fact is at once evident from a consideration of their respective compositions set out in Table VIII, the analysis being effected after hydrofluoric acid treatment or fusion with alkalis to decompose the silicates completely. Of the fine sand (0·2 to 0·4 mm. diameter) 95 per cent. is silica ($SiO_2$), but ferric oxide, alumina and potash are invariably present; the silt (0·04 to 0·01 mm. diameter) contains rather less silica—88 per cent.—and more alumina and potash. The fine silt was known to be a mixture and was therefore fractionated; the portion 0·01 to 0·005 mm. is of the same character though the silica continues to fall to 84 per cent., but the finer fraction, 0·005 to 0·002 mm. diameter, shows an abrupt change in composition, the silica suddenly dropping to 65 per cent. while the alumina, ferric oxide and potash rise to about 18, 8 and 5 per cent. respectively. Probably this latter portion also is a mixture that could be separated by further fractionation, but its proportion in the soil is usually so small that the separation is without practical significance. The clay is clearly a complex silicate or a mixture of several, and in our soils two varieties were found: the Gault, Bargate, Brick Earth and Thanet Beds—all highly fertile soils—yield a clay containing about 21 per cent. of alumina and 51 to 54 per cent. of silica, while the Weald, Lower Weald and Clay-with-Flints—all second-rate soils—yield a clay containing about 30 per cent. of alumina and 50 per cent. of silica.

Thus we can make two rough divisions of the soil fractions: the clay and the coarser material. We cannot as yet go any further; the subdivisions of the coarse material into silt, fine sand and sand must be regarded as a purely arbitrary process.

The properties of these fractions have therefore no quantitative character; on our present methods it is not possible to give quantitative expression to the complete mechanical analysis by multiplying the amount of any group by a factor representing its effect. Qualitative interpretations alone are possible as yet.

Again, one of the chief functions of the soil is to regulate the water-

## TABLE VIII.

*Composition of the fractions obtained by mechanical analyses.*

|  | A. Fine sand (0·2 to 0·04 mm. diameter) | | | | | B. Silt (0·04 to 0·01 mm. diameter) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Formation | Gault | Bargate | Brick earth | Rothamsted clay-with-flints | Av. | Gault | Bargate | Brick earth | Rothamsted clay-with-flints | Av. |
| No. of soil | 217 | 202 | 207—212 |  |  | 217 | 202 | 207—212 |  |  |
| $SiO_2$ | 94·3 | 95·9 | 91·1 | 94·6 | 94·0 | 88·7 | 88·6 | 88·4 | 92·0 | 89·4 |
| $Al_2O_3$ | 0·8 | 1·0 | 2·7 | 3·4 | 2·0 | 5·4 | 2·5 | 6·3 | 6·2 | 5·1 |
| $Fe_2O_3$ | 1·1 | 1·2 | 1·2 | 1·1 | 1·2 | 1·2 | 2·0 | 1·6 | 1·2 | 1·5 |
| CaO | 0·7 | 0·3 | 0·4 | — | 0·5 | 1·1 | 0·4 | 0·8 | — | 0·8 |
| MgO | 0·1 | 0·1 | 0·1 | — | 0·1 | 0·3 | 0·4 | 0·2 | — | 0·3 |
| $K_2O$ | 1·1 | 0·8 | 2·5 | — | 1·5 | 2·6 | 1·6 | 2·7 | — | 2·3 |
| $P_2O_5$ | 0·1 | 0·1 | 0·1 | — | 0·1 | 0·1 | 0·1 | 0·1 | — | 0·1 |
| Undetermined | 1·8 | 0·6 | 1·9 | — |  | 0·6 | 4·4 |  |  |  |
| Total | 100·0 | 100·0 | 100·0 | — |  | 100·0 | 100·0 | 100·0 |  |  |

### C. Fractions of the fine silt.

|  | A. The coarser part (0·01 to ·005 mm. diameter) | | | | | B. The finer part (0·005 to 0·002 mm. diameter) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Formation | Gault | Bargate | Brick earth | Rothamsted clay-with-flints | Av. | Gault | Bargate | Brick earth | Rothamsted clay-with-flints | Av. |
| No. of soil | 217 | 202 | 207—212 |  |  | 217 | 202 | 207—212 |  |  |
| $SiO_2$ | 79·5 | 84·2 | 84·4 | 88·3 | 84·1 | 68·4 | 66·9 | 60·3 | 61·7 | 64·3 |
| $Al_2O_3$ | 4·9 | 7·7 | 5·8 | 8·5 | 7·2 | 16·1 | 16·0 | 21·5 | 23·4 | 19·3 |
| $Fe_2O_3$ | 2·5 | 3·3 | 2·8 | 1·8 | 2·6 | 6·5 | 9·6 | 7·4 | 7·0 | 7·6 |
| CaO | 2·2 | 0·6 | 0·5 | — | 1·1 | 1·9 | 2·8 | 1·8 | — | 2·2 |
| MgO | 0·3 | 0·1 | 0·2 | — | 0·2 |  | 0·3 | 0·4 | — | 0·4 |
| $K_2O$ | 2·8 | 1·7 | 5·0 | — | 3·2 | 1·9 | 5·7 | 8·2 | — | 5·3 |
| $P_2O_5$ | 0·1 | 0·1 | 0·1 | — | 0·1 | 0·4 | 0·5 | 0·3 | — | 0·4 |
| Undetermined | 7·7 | 2·3 | 1·2 | — |  | 4·8 | — | 0·1 |  |  |
| Total | 100·0 | 100·0 | 100·0 |  |  | 100·0 | 101·8 | 100·0 |  |  |

### D. The clay.

|  | Group 1. Fertile soils | | | | | Group 2. Less fertile soils | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Formation | Gault | Bargate | Brick earth | Thanet | Av. | Weald | Lower weald | Clay-with-flints | | Av. |
| No. of soil | 217 | 202 | 207—212 | 118 |  | 70 | 244 | 159 | Roth d |  |
| $SiO_2$ | 54·4 | 54·5 | 50·9 | 53·2 | 53·2 | 49·6 | 50·5 | 48·6 | 47·2 | 49·0 |
| $Al_2O_3$ | 21·3 | 20·8 | 22·8 | 21·2 | 21·5 | 27·2 | 32·1 | 29·6 | 30·1 | 29·8 |
| $Fe_2O_3$ | 11·8 | 13·9 | 13·1 | 13·8 | 13·2 | 12·1 | 11·8 | 16·2 | 12·4 | 13·1 |
| CaO | 1·7 | 1·7 | 2·3 | 0·5 | 1·6 | 0·8 | 0·7 | 1·1 | 3·6 | 1·5 |
| MgO | 0·9 | 0·6 | 1·0 | 1·6 | 1·0 | 0·5 | 0·6 | 1·3 | 1·4 | 1·0 |
| $K_2O$ | 2·7 | 5·9 | 7·2 | 3·9 | 4·9 | 4·1 | 3·9 | 3·3 | 2·5 | 3·4 |
| $P_2O_5$ | 0·1 | 0·6 | 0·4 | 0·5 | 0·4 | 0·3 | 0·5 | 0·6 | 1·6 | 0·7 |
| Undetermined | 7·1 | 2·0 | 2·3 | 5·3 | 4·2 | 5·4 | — | — | 1·2 |  |
| Total | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·1 | 100·7 | 100·0 | — |

supply, and, as the soil possesses no power of adaptation, the nature and amount of the water-supply is fully as important as the nature of the soil. A set of figures bearing one interpretation in a wet district would bear quite another under drier conditions; indeed one can go so far as to say that a mechanical analysis can only be fully interpreted in terms of the water-supply. It is, of course, not the properties of the fractions that change as the water-supply changes, but the relative importance of the part they play in crop production.

Bearing these limitations in mind the following properties will be found useful in interpreting a mechanical analysis. The clay fraction (below 0 002 mm. in diameter) is perhaps the most important in determining fertility. It has the effect of binding a soil, of increasing the water-holding capacity and thus to a certain point of improving the water-supply. If only a small amount is present the soil has so little power of storing water that the plant is entirely dependent on a regular external supply such as frequent showers or underground water near the surface. In absence of these special conditions soils containing less than 4 per cent. of clay are, the south-east of England, barren and uncultivated. With rather more clay the water-supply is still precarious, but may be improved by dung or organic manures, and then the special properties of the other soil fractions enable the soil to be used for market garden purposes. When the clay rises to 10 or 14 per cent. very satisfactory results are obtained; the soil is now a good loam, sufficiently retentive of water and yet not difficult to work; usually also its air supply and temperature relationships are satisfactory. Still larger amounts of clay—20 per cent. or more—have too great a binding effect; unless this is counteracted by the presence of sufficient coarse sand, organic matter or calcium carbonate, the soils become difficult to work, especially in wet districts, and tend to go down in grass. As the clay rises still higher plant growth becomes increasingly difficult; the ground tends to parch and to crack badly in summer, and to lie very wet in winter. Good pastures in the districts we have examined are rarely found on soils containing 30 per cent. of clay, whilst if as much as 40 per cent. is present the land is not generally in cultivation. We have never found a soil or subsoil containing more than 50 per cent. of clay.

Fine silt (0·01 to 0·002 mm. in diameter), like clay, improves the water-holding power of the soil by reason of its small dimensions and its relatively large surface; it also increases the stickiness and difficulty of working the soil, especially if much clay is present. But it differs

fundamentally from clay in one important respect. Clay possesses all the properties of colloids in addition to plasticity; while fine silt does not show colloidal properties. Thus fine silt cannot be flocculated by lime like clay, indeed no method is known for making it tractable. In consequence soils containing 15 per cent. or more are difficult to work, and they tend to go down to rather poor grass if 20 per cent. is present; large amounts of organic matter, however, mask the objectionable properties. There is usually less fine silt than clay. In the few cases where the reverse obtains some peculiarities show themselves in cultivation; such soils occur in the Lower Wealden strata, in Upper Greensand, and among the warp lands of Lincolnshire.

The coarser grade of silt (0·04 to 0·01 mm. in diameter) appears to be very valuable and forms the chief constituent of many highly fertile soils in the south-east of England, from 30 to 40 per cent. being found in some of the loams most famous for carrying their crops well and not drying out. Light sandy loams, on the other hand, may contain only 10 to 20 per cent.; some of these are highly fertile but as a rule they require large dressings of dung or else a favourable situation for water-supply. Probably silt plays a very important part in maintaining the even conditions of moistness so desirable for plant growth. It is fine enough to retard, but not to prevent, the downward movement of water, and it facilitates capillary movement.

Fine sand (0·2 to 0·04 mm. in diameter) forms a considerable fraction of nearly all soils; only rarely is there less than 10 per cent., while often there is as much as 20 per cent. or even more. Although its dimensions are relatively large it still possesses cohesiveness and tends to cake together; it has not, however, so great an effect as silt in maintaining a good moist condition. Soils containing much fine sand— 40 per cent. or more—tend to form after rain a hard crust on the surface through which young plants can only make their way with difficulty until it has been broken by a roller. But they have no great water-holding capacity or retentive power and are not infrequently described by their cultivators as hungry soils that cannot stand drought. The notoriously infertile Bagshot sands and the barren Hythe Beds in West Surrey, are largely composed of this fraction, as much as 70 per cent. sometimes being present. In all these cases, however, clay is deficient, and the situation is dry; better results are obtained with them when the clay exceeds 8 or 9 per cent., or when the water table is near the surface, especially if the amounts of coarse sand and gravel are not too high.

Coarse sand is perhaps the most variable in amount of all soil constituents, and, as its properties are in many ways the reverse of those of clay, it exercises a very great effect in determining fertility. It keeps the soil open and makes it friable; in moderate amounts it facilitates working, but when the amount becomes too high it increases the drainage and evaporation so much as to interfere seriously with the water-holding capacity of the soil. Many good loams contain less than 4 per cent., and generally strong or tenacious soils contain less coarse sand than one-half the quantity of clay present. When the coarse sand exceeds the clay in amount the soil becomes light unless of course the clay is above 20 per cent. when the soil must always remain heavy. Soils containing 40 per cent. or more of coarse sand and less than 5 per cent. of clay are only cultivated where large quantities of dung are available or where the water-supply is exceptionally good. As the amount of coarse sand increases the soils become less and less suited to cultivation till finally the sand dune condition is reached, when over 95 per cent. is present.

Fine gravel is not usually present to any great extent, and is of importance only when the coarse sand is already dangerously high. Stones cannot be determined quantitatively by any method of sampling in use, and their effect must be judged by a visit to the field. If they are uniformly scattered through a stiff soil, as in the Clay-with-Flints, they are on the whole beneficial because they facilitate tillage. Where they form a bed underlying the soil they may do harm by causing over-drainage.

A few illustrations are given in Table IX to show how these properties may be applied in discussing particular analyses; they are all taken from our survey of Kent, Surrey and Sussex.

The Chilworth soil contains so little clay and fine silt and so much coarse sand, that it has very little power of retaining water. As it lies too high to obtain any seepage water from the neighbouring formations it is dependent on the immediate rainfall, and is therefore not in cultivation but has always been heath land. Owing to its bad constitution and its high situation it could not by any known method be made suitable for farming.

The Shalford soil lies lower down and has a better water-supply, less coarse sand and more clay and fine silt. But its water-holding capacity and its retentive power for manures are still very low; artificial manures are of much less value than organic manures, and the best treatment of the land is to grow green crops and fold them off to sheep.

It is better suited for special purposes like market garden crops than for ordinary mixed farming.

TABLE IX.

*Mechanical analyses of soils and their interpretations.*

| Formation... | Folkestone beds | | Thanet beds | | Brick earth | | London clay | Weald clay | Alluvium | |
|---|---|---|---|---|---|---|---|---|---|---|
| Locality...... | Chil-worth | Shal-ford | Gold-stone | Bar-ton | Ick-ham | Oving | Tol-worth | Shaddox-hurst | Ewhurst | |
| | 170 | 124 | 678 | 118 | 129 | 211 | 107 | 267 | 174 | 285 |
| Gravel ...... | 1·2 | 2·5 | 0·2 | 0·2 | 0·3 | 0·9 | 0·4 | 0·2 | 0·7 | 0·1 |
| Coarse sand | 65·9 | 52·6 | 15·3 | 2·3 | 0·7 | 1·3 | 12·8 | 1·5 | 1·0 | 0·5 |
| Fine sand ... | 23·7 | 26·2 | 44·9 | 34·7 | 24·7 | 16·0 | 25·5 | 11·0 | 19·8 | 19·3 |
| Silt............ | 2·4 | 4·8 | 17·3 | 36·2 | 44·8 | 35·5 | 11·3 | 19·6 | 28·4 | 13·0 |
| Fine silt...... | 2·0 | 3·5 | 6·3 | 6·3 | 8·6 | 13·3 | 11·1 | 26·8 | 12·1 | 20·0 |
| Clay ......... | 0·9 | 3·8 | 8·9 | 11·5 | 14·7 | 15·9 | 23·7 | 22·1 | 19·7 | 26·9 |

The Goldstone soil is more productive and more generally useful. It possesses more clay and fine silt than the two previous soils and has therefore better power of retaining water and manures. But it is still distinctly light and is made more so by the circumstance that the coarse sand exceeds the clay in amount; it responds better to organic than to artificial manures and suffers rather in droughty weather in spite of lying not far above the marshes. It contains 45 per cent. of fine sand and therefore tends to cake on the surface after rain and to form steely lumps if worked when wet. Under proper management, however, it produces good crops and is equally suited for ordinary arable and for fruit or potato cultivation.

The next three soils may be taken as illustrations of the very best loams in the district. Silt forms the largest fraction and therefore the soils possess sufficient but not too great a power of retaining water. The clay varies between 12 and 16 per cent., a very satisfactory amount provided the rainfall is not too high. The fine silt is always lower. As there is a considerable amount of fine sand and no excess of fine silt and clay the absence of coarse sand is no disadvantage.

The Tolworth soil is highly productive arable land but almost too heavy for profitable cultivation; only by dressings of dung, which can fortunately be got cheaply from town, can it be kept workable. It

contains rather too much clay and would no doubt have gone down to grass had there not been so much coarse sand present.

The Shaddoxhurst soil is bad. It contains a large amount of clay and a still larger amount of fine silt, consequently its texture is not improved as much as might be expected by liming. There is practically no coarse sand and not even very much fine sand to keep the soil open, it has deservedly always been in bad repute. It is best as pasture land, and then requires drainage and treatment with basic slag; it may be made useful, but never first rate.

The Ewhurst soils are both in pasture, being too heavy for arable cultivation on account of their high clay and low coarse sand content. No. 174 has the better constitution; silt is the predominant feature, the clay is not too high nor is the fine silt. It has all the characteristics of a good, if heavy, soil, and is indeed known to be an excellent bullock pasture. No. 285, on the other hand, is not so good; it contains too much clay and fine silt, and too little silt and coarse sand. It has no great agricultural value.

As already pointed out, it is not yet possible to predict the behaviour of a soil with regard to particular crops. We have, however, made a careful study of a number of soils known to be favourable to certain crops; the analyses are set out in Table X.

Among the wheat soils 63 would perhaps be rather light were it not well supplied with subsoil water: 67 at the other end of the series is now too heavy for profitable cultivation although it was at one time famous for wheat. The intermediate soils form pre-eminently the wheat soils of the district, and appear to represent the ideal mixture of particles for the prevailing climatic conditions—a rainfall of some 26 to 30 inches and a warm summer and autumn. But little gravel and coarse sand are present to keep the soil open, though the fine sand and silt, the most abundant fractions, permit of some movement of water. The clay and fine silt are sufficient in amount to impart firmness to the soil, without being large enough to make the soil unworkable. Wheat, however, possesses considerable power of adaptation and is less localised than barley, potatoes, fruit or hops.

Chemical analysis of these soils revealed no connection between their chemical composition and their suitability for wheat.

The barley soils are distinctly lighter in character. They usually contain a fair amount of coarse sand, rising to 50 per cent. in the wetter districts from which 124 and 203 were taken, though the well-drained soil 207 (see p. 214) contains less than 1 per cent. The fine sand is

usually the largest constituent of the soil, being about 30 or 40 per cent., the fine silt is rather low, and the clay, except in 149 and 180 $a$, is about 10 per cent.

The potato soils are still lighter, and are indeed the lightest of all soils in cultivation, probably in part because potatoes are worked in with market garden produce rather than ordinary farm-crops. They differ from the barley soils in their distinctly lower proportions of silt and of clay, and their larger content of coarse sand. As in the barley soils the chief constituent is fine sand.

The different varieties of hops show some differences in their soil requirements, the finest sorts, Goldings and Bramlings, grow on the deep moist loams 193 to 129, 193 being, however, rather light and more suited for fruit. Fine sand and silt make up about 65 per cent. of these soils; the fine silt is low and the clay is about 12 per cent. In comparison with the barley soils the coarse sand is lower, and the fine silt and silt together are higher; there is no doubt, however, that excellent barley could be grown on these soils, but hops are considered to be more profitable.

Soils 179 to 172 are unsuited to the finer varieties but give excellent crops of the coarser Fuggles, Cobbs, etc. They are heavy loams of the rather peculiar type associated with the Wealden and Lower Wealden Beds.

Fruit soils on the whole are lighter than hop soils, but there is considerable overlapping: the Brick Earth and the Ragstone grow both fruit and hops well, but there are many soils just too light for hops but good for fruit, while at the other end of the scale are soils that grow hops fairly but are too heavy for fruit. The different sorts of fruit have their own requirements; strawberries, red currants and raspberries prefer a light soil, while gooseberries, plums and apples require a free-working loam possessing some consistency. Cherries in addition seem to require a sufficiently pervious subsoil and chalk at no great distance below. On heavy loams apples and black currants will still continue to flourish, but on anything approaching a clay many varieties of apple begin to canker, and all are slow of growth and yield comparatively small-sized fruit.

On the typical fruit soils fine sand is seen to be the chief constituent, followed by silt which together make up about 60 per cent. of the soil. There is some 12 per cent. of clay, but only small quantities of fine gravel and coarse sand, and not much fine silt. In the case of fruit and also of hops other considerations besides soil come into play,

and it by no means follows that every soil of the typical composition would give profitable crops.

Commons and waste lands are almost always found on the lightest sands particularly when the elevation is high. Occasionally commons are found on clay formations where the soil is very heavy and wet; in general, however, this type of soil has at one time or another been brought into cultivation and any that still remains waste does so because some accident prevented it being enclosed. If it were considered necessary this land might still be reclaimed.

Waste soils are characterised by two properties: presence of large amounts of coarse sand or of coarse sand and fine sand, and of small amounts only of clay and fine silt. Almost invariably calcium carbonate is absent; many of the soils have further an acid reaction.

Such, then, are the facts revealed by analysis of soils of known vegetation relationships, we can now proceed to ascertain more precisely their meaning by studying in still further detail the interpretation of a mechanical analysis.

TABLE X.

*Mechanical analyses of soils well suited to certain crops.*

A.  *Wheat soils.*

| Formation ... | Thanet sand | Upper green-sand | Brick earth | Clay-with-flints | Clay-with-flints | London clay | Alluvium | Weald clay | London clay | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil ... | 63 | 84 | 211 | 180 a | 111 | 107 | 143 | 69 | 67 | |
| Fine gravel... | 1·2 | 5·9 | 0·9 | 1·1 | 1·7 | 0·4 | 0·1 | 0·5 | 0·5 | 1·4 |
| Coarse sand | 5·2 | 4·8 | 1·3 | 1·0 | 5·7 | 12·8 | 0·0 | 2·5 | 0·3 | 3·7 |
| Fine sand ... | 32·1 | 26·5 | 16·0 | 30·7 | 26·5 | 25·5 | 31·1 | 14·7 | 17·6 | 24·5 |
| Silt ............ | 33·3 | 25·9 | 35·5 | 24·9 | 20·5 | 11·3 | 18·1 | 24·2 | 13·4 | 23·0 |
| Fine silt ...... | 7·4 | 12·9 | 13·3 | 9·4 | 9·6 | 11·1 | 12·9 | 23·7 | 15·3 | 12·8 |
| Clay ......... | 11·9 | 13·2 | 15·9 | 18·7 | 20·0 | 23·7 | 19·7 | 20·1 | 36·8 | 20·0 |

B.  *Barley soils.*

| Formation ... | Lower green-sand | Lower green-sand | Thanet sand | Lower green-sand | Thanet sand | Brick earth | Chalk | Lower green-sand | Clay-with-flints | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil ... | 124 | 203 | 678 | 122 | 63 | 207 | 61 | 149 | 180 a | |
| Fine gravel... | 2·5 | 1·1 | 0·2 | 2·5 | 1·2 | 0·6 | 0·6 | 0·9 | 1·1 | 1·2 |
| Coarse sand | 52·6 | 50·0 | 15·3 | 13·9 | 5·2 | 0·8 | 8·8 | 16·8 | 1·0 | 18·3 |
| Fine sand ... | 26·2 | 20·2 | 44·9 | 44·6 | 32·1 | 25·0 | 35·2 | 28·7 | 30·7 | 32·0 |
| Silt ............ | 4·8 | 6·7 | 17·3 | 14·1 | 33·3 | 27·3 | 25·5 | 9·5 | 24·9 | 18·2 |
| Fine silt ...... | 3·5 | 6·6 | 6·3 | 6·6 | 7·4 | 16·4 | 6·7 | 9·5 | 9·4 | 8·0 |
| Clay ......... | 3·8 | 9·7 | 8·9 | 9·5 | 11·9 | 11·1 | 14·6 | 18·8 | 18·7 | 11·9 |

## TABLE X (continued).

C. *Potato soils.*

| Formation ... | Thanet | Lower green sand | Bagshot | Thanet | Bagshot | Thanet | Chalk | London clay | Mean |
|---|---|---|---|---|---|---|---|---|---|
| No. of soil ... | 181 | 102 | 90 | 64 | 104 | 659 | 62 | 105 | |
| Fine gravel... | 1·2 | 2·9 | 0·1 | 0·5 | 0·7 | 0·3 | 0·5 | 0·6 | 0·9 |
| Coarse sand | 10·2 | 46·6 | 29·5 | 15·0 | 24·8 | 2·0 | 16·0 | 16·9 | 20·1 |
| Fine sand ... | 58·6 | 22·9 | 47·5 | 48·9 | 38·6 | 68·1 | 31·8 | 31·2 | 43·5 |
| Silt ............ | 13·3 | 3·5 | 5·3 | 15·2 | 11·2 | 3·6 | 21·4 | 14·7 | 11·0 |
| Fine silt...... | 5·1 | 8·8 | 5·8 | 5·4 | 6·2 | 4·8 | 5·9 | 8·8 | 6·4 |
| Clay ........ | 5·5 | 6·9 | 7·1 | 9·3 | 9·9 | 11·6 | 12·6 | 14·9 | 9·7 |

D. *Hop soils.*

| | Finer varieties | | | | | | | | Coarser varieties | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Formation ... | Thanet sand | Alluvial | Thanet sand | Brick earth | Hythe beds | Clay-with-flints | Brick earth | Mean | Hastings beds | Weald clay | Hastings beds |
| No. of soil ... | 193 | 140 | 118 | 133 | 127 | 180 b | 129 | | 179 | 69 | 172 |
| Fine gravel... | 0·5 | 3·2 | 0·2 | 0·7 | 2·3 | 0·6 | 0·3 | 1·2 | 0·4 | 0·5 | 2·9 |
| Coarse sand | 16·9 | 13·1 | 2·3 | 2·1 | 9·5 | 0·8 | 0·7 | 4·8 | 0·5 | 2·5 | 1·8 |
| Fine sand ... | 57·3 | 40·0 | 34·7 | 39·1 | 30·6 | 33·7 | 24·7 | 33·8 | 24·7 | 14·7 | 13·2 |
| Silt ............ | 8·2 | 16·5 | 36·2 | 26·1 | 19·7 | 29·3 | 44·8 | 28·8 | 30·1 | 24·2 | 20·4 |
| Fine silt...... | 3·9 | 9·0 | 6·3 | 8·6 | 11·1 | 8·5 | 8·6 | 8·7 | 19·7 | 23·7 | 22·4 |
| Clay ........ | 6·0 | 9·1 | 11·5 | 11·7 | 13·3 | 13·0 | 14·7 | 12·1 | 14·9 | 20·1 | 25·1 |

E. *Fruit soils.*

| Formation ... | Bagshot sand | Thanet sand | Thanet sand | Brick earth | Hythe beds | Chalk | Mean | Clay-with-flints | Hastings beds | Weald clay |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil ... | 88 | 181 | 81 | 120 | 127 | 61 | | 137 | 179 | 43 |
| Fine gravel... | 0·1 | 1·2 | 0·6 | 0·3 | 2·3 | 0·6 | 1·0 | 1·2 | 0·4 | 2·0 |
| Coarse sand | 17·1 | 10·2 | 4·7 | 0·8 | 9·5 | 8·8 | 6·8 | 1·4 | 0·5 | 3·6 |
| Fine sand ... | 66·4 | 58·6 | 55·2 | 30·2 | 30·6 | 35·2 | 42·0 | 34·0 | 24·7 | 10·6 |
| Silt ............ | 3·5 | 13·3 | 14·1 | 43·7 | 19·7 | 25·5 | 23·3 | 25·4 | 30·1 | 12·8 |
| Fine silt...... | 3·9 | 5·1 | 5·7 | 7·8 | 11·1 | 6·7 | 7·3 | 10·2 | 19·7 | 22·0 |
| Clay ........ | 3·6 | 5·5 | 10·9 | 10·4 | 13·3 | 14·6 | 10·9 | 14·4 | 14·9 | 33·8 |
| | Only Nursery stock | Straw-berries | Mixed fruit | Mixed fruit | Mixed fruit | Mixed fruit | | Cherries, Apples | Apples, Black currants | Apples bad* |

* This soil is too heavy for fruit, though apple orchards are found upon it.

TABLE X (continued).

F. Wastes and Commons.

| Formation ... | Folkestone beds | | | | Bagshot beds | Hythe beds | Oldhaven beds | Ashdown beds | London clay | Claywithflints |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unsuited for cultivation | | | | | | | Uncultivated for accidental reasons | | |
| No. of soil ... | 14 | 171 | 32 | 192 | 91 | 168 | 76 | 241 | 57 | 157 |
| Fine gravel... | 0·1 | 1·2 | 2·1 | 0·8 | 0·7 | 4·2 | 6·8 | 0·2 | 0·1 | 0·5 |
| Coarse sand... | 68·5 | 65·9 | 37·7 | 59·7 | 16·6 | 12·7 | 12·8 | 0·3 | 5·5 | 0·5 |
| Fine sand...... | 18·1 | 23·7 | 47·6 | 22·1 | 64·2 | 51·6 | 36·1 | 53·2 | 23·0 | 33·5 |
| Silt ........... | 4·3 | 2·4 | 3·2 | 3·9 | 7·1 | 10·9 | 16·0 | 19·9 | 14·9 | 32·9 |
| Fine silt ...... | 2·3 | 2·0 | 2·3 | 3·8 | 3·9 | 5·5 | 8·3 | 10·1 | 17·7 | 10·4 |
| Clay ........... | 0·2 | 0·9 | 0·7 | 2·7 | 1·0 | 3·4 | 3·3 | 5·9 | 21·3 | 11·0 |

## FACTORS MODIFYING THE INTERPRETATION OF A MECHANICAL ANALYSIS.

*The amount of organic matter.* Organic matter at the proper stage of decomposition has the effect of binding a loose soil and lightening a heavy one; thus it reduces the difference between a light sand and a heavy clay, bringing them both close to the loams. When there is much organic matter it so impresses its properties on the soil that the mechanical analysis loses much of its significance, and all the analyst can do is to point out what the soil would become if by a persistence in certain methods of management the organic matter were reduced below a certain point.

The prairie soils of Western Canada afford the best illustration of this; they contain so much organic matter that the distinctions indicated by mechanical analysis tend to be obliterated, the difference between sands and clays being far less sharply marked than elsewhere. In mechanical composition some of the famous Manitoban soils are much like our poor Weald soils as seen in Table XI. But the Manitoban soils contain some 20 per cent. of organic matter properly decomposed and uniformly disseminated through the top few feet of soil, and to this they owe their properties, the mineral matter being overpowered and only playing a subsidiary part. The Weald soils, on the other hand, contain 5 to 10 per cent. of organic matter in the top nine inches and only 3 to 6 per cent. lower down; their character is therefore due to the mineral rather than to the organic matter.

A further illustration has been recently published by Gimingham[1], two pasture soils in adjoining fields were found to differ markedly in texture, while in mechanical analysis they were identical. The better textured soil, however, contained 20 per cent. of organic matter while the other contained less than 15 per cent.

It is, however, essential that the organic matter should be properly decomposed. Barren sandy wastes not infrequently contain 5 to 10 or even 15 per cent. of organic matter, but much of it is simply dried bracken or other vegetation that has not broken down and has no value, but rather the reverse, in improving the physical conditions. If the drainage is bad a good deal of peat may form and if the water fills up the soil the condition becomes bad whatever the composition may be.

TABLE XI.

*Effect of organic matter\* on the texture of soils.*

|  | Good texture | Poor texture | Good texture | Poor texture |
|---|---|---|---|---|
|  | Manitoban prairies | Weald clay | (Reported by C. T. Gimingham) | |
| Fine gravel | -- | ·5 | — | — |
| Coarse sand | 1·6 | 1 to 2 | ·6 | ·5 |
| Fine sand | 3·8 | 10 to 12 | 4·3 | 8·4 |
| Silt | 17·1 | 20 to 30 | 11·2 | 13·8 |
| Fine silt | 28·2 | 25 to 30 | 28·7 | 26·5 |
| Clay | 23·3 | 20 to 25 | 23·8 | 25·0 |
| Loss on ignition | 26·3 | 5 to 8 | 19·8 | 14·5 |

\* Measured by the loss on ignition.

*The amount of calcium carbonate.* Two soils of similar mechanical composition may behave very differently if they contain dissimilar amounts of calcium carbonate. This is well illustrated in the Hamsey Green and the Rothamsted soils shown in Table XII. In each case one soil is much heavier and stickier than the other, despite the similarity in mechanical composition, and has gone down to grass while the other has remained arable. Chalking used in each case to be the recognised mode of treatment, but for some reason less was applied on one field than on the other, causing the marked differences that now exist.

In interpreting a mechanical analysis it must therefore be remembered that 1 or 2 per cent. of calcium carbonate may greatly

[1] C. T. Gimingham, *Journal of the Board of Agriculture*, 1910, **17**, 529.

modify the clay properties. When the percentage rises much higher and the soil becomes very chalky then the mechanical analysis loses its value, just as it does when much organic matter is present.

<div align="center">

TABLE XII.

*Effect of calcium carbonate on the texture of soils.*

</div>

| | Hamsey Green | | Rothamsted | |
|---|---|---|---|---|
| | Arable soil 110 | Too sticky for arable 109 | Arable soil, Barnfield | Too sticky for arable, Geescroft |
| Fine gravel ... ... | 1·7 | 1·6 | 2·4 | 1·8 |
| Coarse sand ....... | 5·3 | 9·5 | 5·5 | 4·9 |
| Fine sand .......... | 28·7 | 22·3 | 20·3 | 27·8 |
| Silt.................. | 26·3 | 25·4 | 24·4 | 25·4 |
| Fine silt........... | 10·2 | 9·9 | 12·7 | 10·6 |
| Clay .............. . | 16·4 | 16·0 | 22·0 | 19·0 |
| Loss on ignition .. | 4·8 | 5·2 | 4·7 | 5·1 |
| Calcium carbonate | 1·02 | ·48 | 3·0 | ·16 |

### WATER-SUPPLY AND THE INTERPRETATION OF MECHANICAL ANALYSIS.

It has already been pointed out that a mechanical analysis can be interpreted and discussed with any degree of completeness only in terms of the water-supply; the rainfall, the coolness of the climate (which, by reducing evaporation losses increases the water-supply), the presence of moving underground water, and the nature of the subsoil all have to be taken into account.

*Effect of the rainfall.* The effect of a high rainfall is to bring into prominence the "sticky" properties of the fine fractions, and to put into the background their water-holding capacity. Thus a light soil under a high rainfall behaves like a heavier soil under a low rainfall; it is as well supplied with water and on the whole behaves in the same kind of way on cultivation. For example soil 245 (Table XIII) is rather lighter than 181, and yet in virtue of its extra rainfall is more useful for farming purposes; indeed 181 is essentially a market garden soil, requiring large dressings of dung for successful cultivation. Soil 196 is physically as good as No. 127 but agriculturally much inferior; owing to the higher rainfall it becomes somewhat too sticky to cultivate profitably and so is in rather poor grass; 127, on the other hand, is from a highly fertile hop garden.

*Coolness of climate.* Soils containing much coarse sand or fine sand that would scorch or burn in a dry warm district may in a cooler district where evaporation is lessened prove very suitable for cultivation. Potato soils afford some good illustrations; potatoes require a light soil but it must be cool and moist. Soil 102 (Table XIII) fulfils these conditions; it is on a slope facing northwards not very far above a stream, and therefore does not quickly dry out, hence it is very good for main crop potatoes. No. 106, on the other hand, although similar in composition, is so placed that it quickly dries and is of much less value. Some of the potato soils of Dunbar, analysed in our laboratory by Mr S. F. Ashby[1], have all the appearance of soils readily drying out, but in their cool climate this property does not show itself to an injurious extent.

TABLE XIII.

*Water-supply and interpretation of mechanical analysis.*

| | Swanley | Stedham | North Chapel | East Fairleigh | Tol-worth | Nut-field | Dunbar | |
|---|---|---|---|---|---|---|---|---|
| | 181 | 248 | 196 | 127 | 106 | 102 | | |
| Fine gravel | 1·2 | 1·4 | 0·9 | 2·3 | 0·6 | 2·9 | 3·0 | 1·0 |
| Coarse sand | 10·2 | 9·3 | 11·4 | 9·5 | 37·8 | 46·6 | 33·8 | 23·7 |
| Fine sand | 58·6 | 68·5 | 43·2 | 30·6 | 33·1 | 22·9 | 28·0 | 38·2 |
| Silt | 13·3 | 3·6 | 13·0 | 19·7 | 7·7 | 3·5 | 5·5 | 6·8 |
| Fine silt | 5·1 | 5·6 | 10·2 | 11·1 | 4·7 | 8·8 | 10·8 | 11·8 |
| Clay | 5·5 | 5·5 | 10·9 | 13·3 | 7·6 | 6·9 | 6·6 | 9·5 |
| Loss on ignition | 2·9 | 3·4 | 5·1 | 5·6 | 3·6 | 3·6 | 6·9 | 6·2 |
| Calcium carbonate | ·02 | ·03 | ·80 | 1·0 | ·27 | ·21 | ·15 | ·31 |
| Rainfall in inches (approximate) | 24 | 33 | 30 | 24 | 28 | 27·5 | 25 | 25 |

So important are rainfall and climatic considerations that no soil survey can be considered complete without a rainfall map and an indication of the cooler and the hotter parts of the region under consideration. Unfortunately rainfall maps on a sufficient scale do not exist and have to be made from the data in *British Rainfall*; these are not as extensive as might be desired.

*Effect of underground water.* When the underground water is near the surface, but at the same time sufficiently far below for proper root development to take place, the most important property of the soil

[1] *Journal of Agricultural Science*, 1905, I. 347.

becomes its power of lifting the water by surface action up to the roots
and so affording them a regular and adequate supply. The silt and
sands are in such cases the useful constituents, the clay and fine silt
being less necessary. Soil 189 (Table XIV), at about three feet
below its surface, has a drifting current of underground water which
is lifted to the roots by the fine and coarse sand. It therefore grows
excellent wheat crops. The Bagshot sands, however, although similar
in physical type, have in general no such water-supplies and are sterile
because they lack the clay which, in their circumstances, could alone
ensure them an adequate power of holding water from one shower to
the next.

<div align="center">TABLE XIV.</div>

<div align="center">*Underground water and mechanical analysis.*</div>

|  | Weybridge | Bagshot beds | Shalford | Lydd |
|---|---|---|---|---|
|  | 189 |  | 124 | 200 |
| Fine gravel ..... | 1·3 | ·1 to ·6 | 2·5 | 0·1 |
| Coarse sand .. | 38·4 | 20 to 30 | 52·6 | 0·9 |
| Fine sand ...... | 39·9 | 45 to 65 | 26·2 | 66·7 |
| Silt ............. | 5·6 | 5 to 10 | 4·8 | 7·2 |
| Fine silt ......... | 5·1 | 5 to 10 | 3·5 | 11·4 |
| Clay .............. | 3·8 | 3 to 7 | 3·8 | 3·9 |

Soil 124 is a light sand with too little power of retaining
water for pastures to go through a hot summer, consequently the
grass-land, except near the brooks, is parched and scorched. No. 200
is certainly somewhat finer grained, but not so very different that
one would expect to find it much better for pasture purposes, yet it
produces one of the best pastures in Romney marsh, not only carrying
but fatting sheep throughout the summer. It has, however, a constant
supply of water three or four feet below the surface, while the water
level is much further below the surface of 124.

This underground flow is one of the factors concerned in the
proverbial fertility of valleys. Soils lying towards the bottom of a long
slope receive not only the rainfall but also the water steadily drifting
downwards to the stream or marsh at the bottom, and this advantage is
further enhanced by the gradual transport of soil down the slope which
increases the depth through which the plant roots can range.

*Effect of the subsoil.* In general the subsoil is rather heavier in type
than the surface soil; in the case of clays it is much heavier; examples

are given in Table XV. The rare exceptions to this rule probably arise through periodical flooding with water containing much clay in suspension or through the occurrence of a bed of sand just below the surface. Although samples are only taken to a depth of 18 inches it is always necessary to know something of the material lying still lower; the water level marks the limit beyond which one need not explore.

There are two cases that call for special notice. A layer of impermeable material may occur near the surface and cause water logging in wet weather and parching in dry weather; or there may be a bed of gravel or stones that causes the soil to be over-drained. The first case is illustrated by soils 152, 219 and 255 in Table XV. No. 152

TABLE XV.

*Nature of the subsoil.*

| | Loddington | | Harting | | Dicker | | Shopwyke | | Wye | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 152 | | 219 | | 255 | | 207 | | 100 | |
| | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil | Surface | Subsoil |
| Fine gravel ...... | 3·5 | 2·6 | 0·6 | 0·2 | 1·0 | 0·6 | 0·6 | 0·1 | 1·0 | 0·2 |
| Coarse sand...... | 10·2 | 9·8 | 3·3 | 3·2 | 2·0 | 1·1 | 0·8 | 0·4 | 3·0 | 1·9 |
| Fine sand......... | 33·5 | 30·2 | 31·6 | 33·9 | 26·6 | 23·2 | 25·0 | 21·9 | 27·2 | 25·3 |
| Silt ... ......... | 14·6 | 17·5 | 17·3 | 21·3 | 23·0 | 15·1 | 27·3 | 38·0 | 40·0 | 41·4 |
| Fine silt ........ | 14·9 | 15·5 | 14·5 | 13·4 | 17·8 | 21·9 | 16·4 | 15·2 | 8·9 | 9·6 |
| Clay ............ | 12·2 | 15·3 | 12·3 | 16·0 | 17·9 | 25·7 | 11·1 | 15·7 | 11·2 | 14·5 |

is typical of an area near Maidstone much of which was waste land. Its sterility was not due to any fault in the soil, which is obviously of excellent type, but to a thin layer of rock lying near the surface. When this was removed a very good soil was obtained. Soil No. 219 lies on the Upper Greensand in West Sussex; the rock comes close to the surface, restricting both the root range of the plant and the water-supply if it lies horizontally, but it does much less harm if it dips at any considerable angle. The soil itself is good although it has rather too much fine silt, it becomes very productive when the effect of the rock is counteracted. No. 255 soil, while not of the best type, as its fine silt is too high, is far from being hopeless, but it unfortunately lies on a deep bed of stiff clay which keeps it wet in winter and parched in summer. It is therefore very poor and even with the best management

is not likely to give great results. Other illustrations are afforded by the not uncommon case of a patch of sand resting in a saucer-shaped depression of clay and thus rendered infertile until a way out is found for the water. A comparatively thin parting of clay may profoundly modify the properties of a sandy soil.

The second case, over-drainage, is illustrated by soil No. 207 in the same Table. This is a fair soil, containing too much fine silt to be in the first rank, and spoiled by lying on a deep bed of gravel which is only nine inches or a foot below the surface, indeed the subsoil sample could only be taken in one or two instances. Consequently it dries out badly in summer and does not repay much expenditure in the way of manures.

Soil No. 100 is given as an instance of the normal case where a soil becomes rather heavier in its lower depths, with the result that the movement of water is somewhat impeded without being stopped. Thus the subsoil furnishes a reserve of water for the surface, yet even in wet weather it does not hold up too much water and in dry weather does not constitute too great a barrier against any upward capillary movement. In marked contrast is the Dicker soil, in which, as usual in clays, the subsoil contains much more true clay than the surface.

### Chemical Analysis of Soils.

The chemical analysis of soil, unlike chemical analysis in general, does not aim at finding out the actual composition of the soil, but only the amounts of various constituents extracted by acids under certain arbitrary conditions. It does not deal with the whole of the soil (except in one or two cases) but only with the less resistant portions, and consequently does not give nearly so complete a picture as the mechanical analysis. The methods are arbitrary and are agreed upon by the majority of chemists in each country, but there is no international agreement, and determinations made here are not necessarily comparable with those made on the Continent, in the United States, or in some of the Colonies. Nor are they comparable with those made by the geologist or mineralogist.

*Organic matter.* It is usual to estimate only the loss on ignition. No method is known for determining the total organic matter and none of the conventions that has been suggested has gained general acceptance. The loss on ignition includes a certain amount of water of combination coming from the clay and perhaps some of the fine silt, etc.,

but it is not yet possible to separate these two quantities. More important than the precise amount of organic matter is its nature; whether sufficiently decomposed, whether acid or not.

Reference has already been made to the inutility of undecomposed organic matter. Acid organic matter or peat is also of little value until it has been frequently treated with lime or basic slag and then it is more useful for certain special crops than for ordinary farming. On the other hand organic matter that is not acid may be extremely valuable, as shown by the prairie soils of Canada and some of the black soils in the Fen country. In the latter case the soils do not respond to lime or to basic slag but give good returns with superphosphate.

*Nitrogen.* Unlike the other soil constituents nitrogen and carbonates are determined absolutely. The amount of nitrogen is closely related to the loss on ignition, of which in a large proportion of cases it is about 3 per cent. As a guide to fertility it is therefore subject to the same limitations; a high nitrogen content may be associated either with a rich soil containing abundance of valuable non-acid organic matter or with a soil where the conditions are so unfavourable that organic debris does not decompose or only forms accumulations of peat. On the other hand some of the best loams, where the conditions are most favourable to rapid decomposition and nitrification, contain but little nitrogen. A few typical examples are given in Table XVI. Soils

TABLE XVI.

*Nitrogen and loss on ignition.*

| | Fertile arable soils | | | | Poor arable soils | | | | Barren wastes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil.............. | 129 | 211 | 193 | 152 | 242 | 255 | 106 | 153 | 197 | 241 | 168 |
| Loss on ignition ...... | 4·65 | 6·58 | 3·70 | 4·65 | 4·13 | 6·23 | 3·60 | 5·14 | 5·94 | 7·00 | 5·81 |
| Nitrogen .............. | ·120 | ·220 | ·133 | ·141 | ·128 | ·143 | ·182 | ·152 | ·130 | ·195 | ·167 |
| Loss on ignition in subsoil } ... | 3·00 | 4·94 | 2·81 | 3·29 | 3·74 | 5·50 | 2·58 | 4·14 | | ---- | 2·70 |
| Nitrogen in subsoil ... | ·078 | ·139 | ·081 | ·097 | ·112 | ·104 | ·061 | ·096 | --- | — | ·058 |

containing much calcium carbonate are as a rule rich in nitrogen, partly no doubt because of the rather high nitrogen content of the rock and partly also because they are folded, green manured, cropped with leguminous plants like sainfoin, lucerne, etc., all of which tend to increase the nitrogen supply.

The nitrogen in some chalk soils is as follows:

| No. of soil | 66 | 62 | 261 | 263 | 269 | 253 |
|---|---|---|---|---|---|---|
| Nitrogen in surface soil | ·25 | ·194 | ·331 | ·258 | ·249 | ·419 |
| ,,    subsoil | ·128 | ·130 | ·162 | ·192 | ·196 | ·180 |
| Calcium carbonate in surface soil ... ... | 18·1 | 3·70 | 49·7 | 66·0 | 65·6 | 44·0 |
| ,,   ,,   ,,   subsoil | 11·37 | 14·9 | 61·3 | 55 2 | 54·8 | 71·6 |

All are arable soils, excepting 253 which is open downland.

*Carbonates.* The smaller the amount of carbonate present the more carefully must the determination be made, especially in sandy soils or where there is much organic matter. Special precautions become necessary to avoid decomposition of organic matter which would give rise to carbon dioxide and thus lead to high results; in our laboratory the treatment of the soil with acid is generally effected in the cold.

The analyst is often asked whether or not a particular soil contains sufficient calcium carbonate, and in endeavouring to answer this question he must bear in mind its twofold function, one to prevent acidity, the other to flocculate the clay. Where only a small amount of clay—say 8 per cent. or less—is present the flocculating action is less needed and it is only necessary to have in the soil sufficient to neutralise any acid that may be found. Soil No. 248 (Table XIII) is an example; it is near the bottom of a slope along which water containing calcium bicarbonate in solution is drifting, and therefore shows no tendency to become acid. The 0·03 per cent. of calcium carbonate present, hopelessly inadequate as it appears, suffices for its needs and no increase in crop is obtained by applying lime. No. 200 contains only ·02 per cent., but is also well supplied with calcareous water from below and therefore shows no sign of acidity. The somewhat similar Red Hill soil, however, has not this advantage of position; and stands in great need of lime although it contains as much as does the Stedham soil. As the amount of clay increases the need for lime becomes greater because the flocculation is now wanted; soils with 20 per cent. or more of clay need two or three times as much lime as sandy soils. It is impossible to fix limits that shall hold universally. Before an analyst recommends lime or chalk on a sandy soil he should satisfy himself that acid indications like finger-and-toe, spurry, etc. are present, and before he states that lime is not necessary on a clay he should be quite sure that further additions would have no beneficial flocculating effect. The following soils were known to respond to lime:

| | Sandy soils | | | Loams and clays | |
|---|---|---|---|---|---|
| No. of soil | Percentage of clay | Percentage of calcium carbonate | No. of soil | Percentage of clay | Percentage of calcium carbonate |
| 126 | 7·8 | ·04 | 207 | 11·1 | ·02 |
| 678 | 8·9 | ·08 | 119 | 10·4 | ·03 |
| 193 | 6·0 | ·18 | 118 | 11·5 | ·18 |
| 189 | 3·8 | ·35 | 152 | 12·2 | ·26 |
| | | | 215 | 13·0 | ·45 |
| | | | 127 | 13·3 | 1·00 |

*Alumina.* In general the alumina determined by extraction with hydrochloric acid is approximately equal in amount to one-third of the clay fraction, and so widely does this rule hold for normal soils that we are justified in supposing that the acid breaks down some definite group of silicates in the soil. The following examples may be quoted:

| | Bagshot sands | | | London clay | | Thanet beds | | Sand-gate beds | Folke-stone beds | Weald clay |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil ...... | 88 | 89 | 90 | 67 | 57 | 118 | 77 | 79 | 102 | 43 |
| Percentage of clay in soil | 3·6 | 4·9 | 7·1 | 36·8 | 21·3 | 11·5 | 7·1 | 15·3 | 6·9 | 33·8 |
| Percentage of $Al_2O_3$ in soil | ·92 | 1·43 | 1·94 | 11·75 | 6·78 | 3·16 | 2·66 | 5·14 | 1·99 | 10·45 |
| Ratio $\frac{Al_2O_3}{clay}$ ...... | ·25 | ·29 | ·27 | ·31 | ·31 | ·30 | ·37 | ·33 | ·28 | ·31 |

Exceptions to the rule occur when much fine silt is present, the alumina in these cases being markedly less than one-third of the clay:

| | Weald clay | | | Lower Wealden beds | | | Upper greensand | | Gault |
|---|---|---|---|---|---|---|---|---|---|
| No. of soil................. | 71 | 52 | 692 | 197 | 244 | 246 | 85 | 219 | 217 |
| Percentage of fine silt in soil | 27·4 | 35·8 | 25·9 | 15·8 | 21·5 | 11·3 | 15·9 | 14·5 | 14·0 |
| Percentage of clay in soil | 21·5 | 22·1 | 19·4 | 5·4 | 12·5 | 9·7 | 13·1 | 12·3 | 11·8 |
| ,, ,, $Al_2O_3$ ,, | 5·02 | 5·42 | 5·68 | ·17 | 1·66 | 2·38 | 2·48 | 2·39 | 5·11 |
| Ratio $\frac{Al_2O_3}{clay}$ .............. | ·23 | ·25 | ·29 | ·03 | ·13 | ·24 | ·18 | ·19 | ·43 |

*Ferric oxide.* The ferric oxide is present in quantities comparable with those of alumina but no close relationship is observable nor does

the amount of ferric oxide afford any indication of the fertility of the soil.   Light soils, good or bad, contain about 1 to 2·5 per cent., good loams and poor clays contain 3·5 to 5 per cent.   None of the soils in our area is rich in ferric oxide and we have therefore been unable to study the effect of quantities larger than 5 per cent.   In the few cases where ferrous iron was present the soils were known not to be of great fertility.

*Lime and magnesia.*   About ·1 to ·5 per cent. of magnesia is found in the soils we have examined, and in general the $\dfrac{\text{lime}}{\text{magnesia}}$ ratio falls between 1 and 3, but ratios of 4 and 5 are not uncommon, while on chalk soils they may rise very high.   We are unable to trace any connection between the $\dfrac{\text{lime}}{\text{magnesia}}$ ratio and the productiveness of the soil, indeed Table XVII shows that very good and very poor soils may have practically identical ratios.

TABLE XVII.

$\dfrac{Lime}{Magnesia}$ *ratio in various soils.*

| | Barren wastes | | | | Poor cultivated soils | | | | Fertile soils | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of soil | CaO | MgO | Ratio $\dfrac{\text{CaO}}{\text{MgO}}$ | No. of soil | CaO | MgO | Ratio $\dfrac{\text{CaO}}{\text{MgO}}$ | No. of soil | CaO | MgO | Ratio $\dfrac{\text{CaO}}{\text{MgO}}$ |
| 170 | ·05 | ·06 | 1·0 | 45 | ·43 | ·23 | 1·9 | 183 | ·56 | ·40 | 1·4 |
| 192 | ·13 | ·08 | 1·6 | 242 | ·30 | ·13 | 2·3 | 222 | ·46 | ·28 | 1·6 |
| 168 | ·21 | ·13 | 1·6 | 106 | ·48 | ·21 | 2·3 | 152 | 1·02 | ·41 | 2·5 |
| 50 | ·15 | ·08 | 1·9 | 255 | ·89 | ·27 | 3·3 | 122 | ·60 | ·22 | 2·7 |
| 197 | ·21 | ·08 | 2·6 | 196 | ·43 | ·12 | 3·6 | 211 | 1·79 | ·40 | 4·5 |
| 91 | ·08 | ·03 | 2·7 | 287 | 1·19 | ·29 | 4·1 | 72 | 1·94 | ·42 | 4·6 |
| 241 | ·22 | ·07 | 3·1 | | | | | 127 | 2·14 | ·40 | 5·4 |
| 13 | ·58 | ·14 | 4·1 | | | | | | | | |

*Potash.*   The amount of potash is closely associated with that of alumina, being commonly about one-tenth, it is therefore about one-thirtieth of the clay.   Some examples are:

| No. of soil | Percentage of $Al_2O_3$ | Percentage of $K_2O$ | Ratio $\dfrac{K_2O}{Al_2O_3}$ | Percentage of clay | Ratio $\dfrac{K_2O}{clay}$ |
|---|---|---|---|---|---|
| 112 | 4·07 | ·45 | ·11 | 13·1 | ·034 |
| 120 | 2·84 | ·31 | ·11 | 10·4 | ·029 |
| 100 | 3·83 | ·40 | ·10 | 11·2 | ·035 |
| 133 | 3·67 | ·44 | ·12 | 11·7 | ·037 |
| 103 | 3·66 | ·30 | ·08 | 11·9 | ·025 |
| 161 | 7·97 | 1·08 | ·14 | 27·7 | ·039 |
| 67 | 11·75 | 1·44 | ·12 | 36·8 | ·039 |
| 118 | 3·46 | ·404 | ·12 | 11·5 | ·035 |
| 79 | 5·14 | ·40 | ·08 | 15·3 | ·026 |
| 43 | 10·45 | ·76 | ·07 | 33·8 | ·022 |
| 147 | 7·88 | ·96 | ·12 | 22·5 | ·043 |

These relationships, which hold for the subsoil as well as the surface soil, can hardly be accidental: they may be taken as an indication that the acid attacks some definite group of silicates associated mainly with the clay fractions.

The potash soluble in 1 per cent. citric acid solution shows no kind of regularity but varies between 5 and 50 per cent. of the quantity extracted by strong acids. In deciding whether or not sufficient is present, attention must be paid not only to the crop but also to the rainfall. In a dry district crops are likely to cease growing and to ripen much earlier than in a moister district. Now potash manures tend to prolong the life of the plant, and in a dry district will keep it growing after it would otherwise have stopped, in consequence they give increased crops. Other things being equal, dry soils are therefore more likely to respond to potassic manuring than others better supplied with water, but no richer in available potash.

Illustrations are afforded by the figures in Table XVIII where soils in the dry eastern part of our district, known to respond profitably to potassic manures, are compared with soils in the western part where the rainfall is much higher and where potassic manures do not prove profitable.

All are arable soils. The chalk pastures on the South Downs usually contain less than ·01 per cent. of available potash, e.g. No. 266, and they respond to potassic manures. It will be observed that ·015 per cent. is insufficient in East Kent where the rainfall is 23 inches, whilst ·010 per cent. suffices in West Sussex under ten inches higher rainfall and generally better water-supply in the soil.

*Phosphoric acid.* Generally speaking the largest amount of phosphoric acid is found in chalk soils, ·2 to ·25 per cent. being present,

about ·15 to 2 per cent. is found in good loams; sandy loams contain about ·1 per cent., while poor clay pastures and poor sands contain still less.    Little if any direct connection can be traced between the phosphoric acid and the productiveness; in general it tends to increase as the clay, fine silt, and silt increase—the poor clay pastures form a readily explained exception—but it does not appear to be closely associated with any one fraction like the potash.  The amounts of available phosphoric acid vary enormously in a region of such highly diversified agriculture as the one now being dealt with; Kentish hop gardens commonly contain ·05 per cent. or more, and we have even found

TABLE XVIII.

*Available potash in soils of known behaviour to potassic manures.*

| | Soils responding to potassic manures | | | | Soils not responding to potassic manures | | | |
|---|---|---|---|---|---|---|---|---|
| | East Kent | Surrey | Sussex | | West Sussex | | | Kent |
| | Newing-ton | Barton | Redhill | Patching | Oving | Rogate | Stedham | Yalding |
| No. of soil...... | 193 | 118 | 126 | 266 | 211 | 222 | 248 | 140 |
| Available K$_2$O | ·013 | ·015 | ·010 | ·007 | ·014 | ·024 | ·010 | ·044 |
| K$_2$O extracted by acid | ·200 | ·404 | ·181 | ·260 | ·43 | ·18 | ·14 | ·59 |
| Clay ............ | 6·0 | 11·5 | 7·8 | 25·5 | 15·9 | 6·7 | 5·5 | 9·1 |
| Rainfall......... | 22·5 | 23 | 27·7 | 28·6 | 28 | 33 | 33 | 24 |

·18 per cent.; well farmed arable soils contain some ·015 per cent., while in the poor worn-out pastures of the Weald the quantity may sink as low as ·002 per cent.   In most cases these quantities are insufficient for some of the crops grown; hop growers regularly apply phosphates [and as they maintain, with profit] to gardens already containing ·05 per cent. of available phosphoric acid, whilst arable farmers use them for swedes even when ·015 per cent. is present.  The exceptions to this rule are to be found on the light soils sufficiently provided with moisture and a forward climate; here the need for phosphates appears to be less.  But in all cases where much purchased food is fed on the land phosphates appear to be of advantage to the succeeding crop.

Rainfall does not appear to have so marked an effect in controlling the need for phosphates as it has for potassic manures.  The explanation

is to be found in the fact that phosphates are useful both in dry and in moist situations; they tend to promote root development, an obvious advantage in a dry soil where the plant will fail unless the roots strike into the deeper, moister layers, they also stimulate the vital processes going on at the end of the season and are thus valuable in wet cold districts. But rainfall and water-supply are important factors in determining the choice of phosphates; basic slag proves less useful than superphosphates on dry soils, and at least as useful on moister soils or under higher rainfall. The amount of chalk in the soil is not the determining factor, but the moistness; if, as often happens, especially in East Kent, a chalky soil is dry, superphosphate will prove the more useful; where, as in Sussex, the soil is moister, basic slag is as good, and of course cheaper.

## The Relative Value of Mechanical and Chemical Analysis.

The fundamental distinction between mechanical and chemical analysis is that the former deals with the whole of the soil, which it sorts out into fractions of varying sizes, while the latter only deals with the part that is readily dissolved by acids. Mechanical analysis therefore gives a complete picture while chemical analysis does not, it is in consequence eminently suited for the purpose of a soil survey, the chief object of which is to classify and describe the soils. Further it enables the investigator to explain with some degree of completeness the observed water relationships of the soil when sufficient is known about the water-supply, and also to account for many of the peculiarities observed in cultivation. It enables him to say, as far as can be said on our present knowledge, whether any observed defects are due to defects in the soil or its situation, or to the system of management that has been adopted. As it cannot be interpreted fully without a knowledge of the amount of organic matter and the calcium carbonate present the two quantities must be determined in every sample.

We have seen that there is a close correlation between the potash, the alumina, and the clay. For purposes of a survey it seems superfluous to determine these two bases in every sample taken. The ferric oxide shows a general correlation with the others but by no means so close; but we have failed to discover any close connection between ferric oxide and fertility in the soils we examined, the ferric oxide being almost always less than 5 per cent. in amount. Nor can we find that the ratio of lime to magnesia is significant. The nitrogen is closely

correlated with the organic matter, *i.e.* the loss on ignition. The total phosphoric acid does not show very great variations in different soils, but the available phosphoric acid, like the available potash, varies greatly with the management. Thus the figures obtained by chemical analysis, apart from the loss on ignition and the calcium carbonate, fall into two groups; the nitrogen, potash, and alumina, which are so closely correlated with quantities already determined in the mechanical analysis that their separate determinate is almost superfluous; and the iron oxide, magnesia, lime, etc. which do not give sufficiently useful indications to be worth determining in every case. Since our present conventional methods of chemical analysis fail to characterise the soil with sufficient completeness we recommend that for purposes of a survey a large number of soils should be submitted to mechanical analysis, including the determination of organic matter and of calcium carbonate, and then a carefully chosen representative set, not necessarily more than a half or even a quarter, should be analysed chemically.

Up to a certain point we agree with Whitney that the mechanical analysis should form the basis of the survey because it alone takes account of those physical functions—the regulation of the water-supply and therefore of the temperature, of the air supply, ease of cultivation, etc.—that play so large a part in determining the value of a soil.

But on the other hand mechanical analysis is restricted in its application and gives useful indications with one only of the well-defined chemical types into which soils may be divided. In one area four chemical groups are recognisable: (1) chalk soils, (2) acid humus or peat soils, (3) neutral humus soils, (4) mineral soils, *i.e.* sands, loams and clays. It is to this last group alone that mechanical analysis can be applied with any degree of usefulness, but since agricultural soils belong so largely to this group the method is really applicable in by far the great majority of cases.

Among the mineral soils there are indications of chemical groups cutting across the mechanical classification, but it is not easy to trace them because so much of every soil is silica. When, however, the clays are separated out and subjected to hydrofluoric acid treatment or fusion with alkalis and then analysed they are seen to fall into two types as shown in Table VIII. Further evidence of dissimilarity among the clays is obtained by a study of the results of the acid extraction of the soil; in general the alumina is about one-third of the clay in amount and the potash is roughly one-tenth of the alumina; in exceptional cases,

however, and usually where the abnormal clay occurs, very different relationships obtain. It will be necessary to accumulate many more analyses of clays before we shall have the material for chemical classifications.

The problem confronting the analyst when a sample of soil is sent in to him is, however, very different from that of a soil survey. In the soil survey the typical soil is being dealt with; the soil sent in by the farmer is usually exceptional and is sent because there is something about it that is not quite what he expects. The analyst has therefore to compare the soil with the typical sample and he must do this both chemically and mechanically. He must of course have a fairly complete knowledge of the typical soil of the particular division of the formation from which the sample comes; in order that this may be at hand information must be obtained during the survey either from farmers or by field experiments that will show the effect of various manures on the typical soil. Further the analyst must study the water-supply, the position and such other factors as affect productiveness and would cause divergencies from the type.

It is because of the great importance of classifying our soils and making a complete study of them that properly conducted soil surveys are needed. The analysis of a single sample of soil casually sent in by a farmer cannot be properly interpreted unless the analyst possesses an adequate knowledge of the type.

# CONTRIBUTIONS TO THE CHEMISTRY OF CHEDDAR CHEESE[1].

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THE " ripening " of cheese consists of a number of chemical changes affecting the fats, proteins and carbohydrates. The protein changes are of the general proteolytic character and are produced by bacteria, fungi, rennet-enzymes and other enzymes formed by bacteria and fungi during their growth in the cheese. The products derived from casein —the main protein-constituent of cheese—are intermediate digestive substances together with aminoacids, and secondary products formed by bacteria from the aminoacids. In a thorough study of the cheese-ripening process it is of fundamental importance to ascertain what specific compounds are formed. Such knowledge must be possessed before the actual working of the process can be fully understood. It is well known to everybody connected with the manufacture of cheese that the quality of the final product depends on the nature of the ripening process.

More or less work of a desultory character has been done on the chemistry of Emmenthaler and American Cheddar cheese, but no work of any scientific importance has so far been undertaken with reference to the well-known Cheddar cheese produced in this country. It will be seen from the investigation, an account of which is given in this paper, that the chemistry of the two previously mentioned cheeses differs considerably from English Cheddar, particularly in the later stages of ripening.

An effective study of the ripening process and its chemical changes demands a separate study

      (1) of the different products formed by each *individual* proteolytic factor (bacteria, fungi, etc.),

      (2) that of their *joint* action.

[1] M. Nierenstein, "The transformation of proteins into fat during the ripening of Cheese," *Proceedings of the Royal Society*, B. **83**, p. 301 [1911].

The present communication deals with the latter part of the question, but investigations on the separate action of bacteria and enzymes are in progress and will form the subject of further communications. The products isolated by me from Cheddar cheese will be discussed under the following separate headings:

 I. Intermediate digestive products.

 II. Aminoacids.

 III. Secondary products formed from aminoacids.

The following general remarks on cheese-formation are given in order that a picture of the process may be obtained. The coagulation of milk by rennet consists of two distinct phases: (1) the formation of casein from caseinogen, and (2) the precipitation of casein by lime salts present in the milk, resulting in the formation of "curd." Soon after the curd-formation has taken place its transformation into three physically different products—$A$, $B$, and $C$ casein—sets in[1]. These three products, for which I propose the name "Cheese-body," form the fresh cheese[2]. Expressed diagrammatically the formation of cheese and its ripening—with reference to the proteins only—would be as follows:

Caseinogen
 ↓
Casein
 ↓  $A$ Casein ⎫
Curd $B$ Casein ⎬ Cheese-body Intermediate digestive products
   $C$ Casein ⎭     Aminoacids
             Secondary products
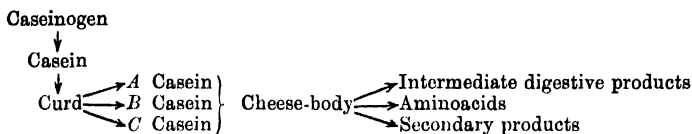
But this cheese-body is not the only source of formation of the different decomposition products, because cheese also contains to a small extent a number of other milk-proteins, such as: lactalbumin, lactoglobulin and opalescin.

## I. *Intermediate digestive products (albumins).*

In 1882 Weidemann[3] called attention to a product which he isolated from Emmenthaler cheese by extraction with boiling alcohol; to this material he gave the name "Caseoglutin." His investigations were continued by Rose and Schulze[4] and Benecke and Schulze[5], who were

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[1] Von Herwerden, *Zeitschr. f. physiolog. Chemie*, **52**, 184 [1907].

[2] The name "Tyrocasein" from the Greek τυρός (cheese) would express my view better; however, as it will be seen further, that name has been adopted by Winterstein for one of the intermediate products formed during the ripening process.

[3] Weidemann, *Landwirtsch. Jahrb.* **11**, 587 [1882].

[4] Rose u. Schulze, *ibid.* **13**, 115 [1884].

[5] Benecke u. Schulze, *ibid.* **16**, 317 [1887].

able to confirm his results. The existence of this intermediate proteolytic product was doubted by van Slyke and Hart[1], who considered it to be "paracasein-lactate"; however, the work of Winterstein[2] and his co-workers Thöni[3] and Bisegger[4] conclusively proved that caseoglutin is formed during the ripening of Emmenthaler cheese. I was also able to prove its existence in Cheddar. For comparison I herewith give the hydrolytic values for caseoglutin obtained by Bisegger from Emmenthaler cheese and by me from Cheddar cheese.

|  | Caseoglutin | |
|---|---|---|
|  | Emmenthaler cheese | Cheddar cheese |
| Histidin | 3·26 °/₀ | — |
| Glutamic acid | 9·53 | 8·65 °/₀ |
| Arginin | 2·27 | 2·60 |
| Lysin | 7·88 | 5·70 |
| Tyrosin | 6·41 | 7·16 |
| Alanin | — | 4·12 |
| Valin | — | 0·26 |
| Leucin | — | 17·08 |

Winterstein[5] also describes a second substance, tyroalbumin, which however I did not succeed in isolating, owing to an oversight. A third product belonging to the albumins is tyrocasein; it is the residue of the cheese-body left after the aminoacids, albumins and secondary products have been removed by successive extraction. Tyrocasein was at first described by Winterstein[6], and its hydrolysis was carried out by his co-worker Bisegger[7]. I have also investigated the residue from Cheddar cheese, and my results agree fairly well with his.

|  | Tyrocasein | |
|---|---|---|
|  | Emmenthaler cheese | Cheddar cheese |
| Histidin | 2·95 °/₀ | 4·26 °/₀ |
| Arginin | 5·84 | 2·16 |
| Lysin | 10·74 | 6·26 |
| Tyrosin | 5·94 | 0·83 |
| Glutamic acid | — | 7·86 |

## II. Aminoacids.

In 1818 Proust[8] discovered in old cheese the substance lysin, which he called "Caseonoxide." In 1860 Sieber[9] also found tyrosin in a sample of Roquefort cheese. The work of Winterstein and Thöni[10]

[1] van Slyke and Hart, Amer. Chem. Journ. 28, 429 [1902] and ibid. 29, 371 [1903].
[2] Winterstein, Zeitschr. f. physiol. Chemie, 41, 485 [1904].
[3] Winterstein u. Thöni, ibid. 36, 28 [1902].
[4] Bisegger, Dissertation, Zurich [1907].
[5] Winterstein, l.c.       [6] Winterstein, l.c.       [7] Bisegger, l.c.
[8] Proust, Ann. Chim. et Phys. [2], 10, 40 [1818].
[9] Sieber, Jour. prakt. Chemie [2], 21, 213 [1880].
[10] Winterstein u. Thöni, Zeitschr. f. physiol. Chemie, 36, 28 [1902].

is of great importance. In 1902 they isolated from Emmenthaler cheese histidin, arginin, and lysin. The latter product had previously been discovered by Steinegger[1] in Emmenthaler cheese. Winterstein added to this list, in a second communication, phenylalanin, glutamic acid, aspartic acid and α-pyrrolidin carboxylic acid. The following table gives the hydrolytic products of caseinogen (I), the products isolated by Winterstein[2] and his co-workers from Emmenthaler cheese after six months' ripening (II), and the products I have obtained from a Cheddar cheese about four years old (III).

|  | Caseinogen I | Emmenthaler cheese II | Cheddar cheese III[3] |
|---|---|---|---|
| *Glycin ... ... ... | 0 % | Present | 0·4 % |
| Alanin ... ... ... | 0·9 | Present | 0·31 |
| *Valin ... ... ... | 1·0 | — | 0·17 |
| *Leucin ... ... ... | 10·5 | Present | 0·12 |
| *Isoleucin ... ... ... | — | Present | 0·32 |
| Phenylalanin ... ... | 3·2 | 2·41 % | Present |
| Tyrosin ... ... ... | 4·5 | 0·58 % | 3·8 |
| Serin ... ... ... | 0·23 | Present | Present |
| Cystin ... ... ... | 0·06 | Absent | — |
| Prolin ... ... ... | 3·1 | — | — |
| Oxyprolin ... ... ... | 0·25 | — | — |
| Aspartic acid ... ... | 1·2 | — | Absent (?) |
| Glutamic acid ... ... | 11·0 | 0·32 % | About 0·7 |
| Tryptophane ... ... | 1·5 | Present | Present |
| Arginin ... ... ... | 4·84 | Absent | Absent |
| Lysin ... ... ... | 5·80 | Present | 0·85 |
| Histidin ... ... ... | 2·59 | 1·23 % | — |
| Diaminotriotydodecanic acid | 0·75 | — | — |
| Aminobutyric acid ... ... | — | Present | 0·43 |
| *Aminovaleric acid ... ... | Present | — | 0·14 |

This table shews that nearly all the aminoacids found in the hydrolysis of caseinogen are met with in the ripened cheese as *free* proteolytic products.

The presence of glycin in the ripened cheese is of great interest, since it has not been so far definitely proved to be present in caseinogen. Emil Fischer[4] found on hydrolysing caseinogen with hydrochloric acid traces of a crystalline product which he considered in all probability to be glycin, but about which he says: "da aber die Menge im ganzen recht geringe war, so halte ich es für wahrscheinlich, dass dieses Gly-

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[1] Steinegger, *Landwirtsch. Jahrb.* **15**, 132 [1886].

[2] Winterstein, *Zeitschr. f. physiol. Chemie*, **41**, 485 [1904].

[3] All those aminoacids which I have proved for the first time to be present in cheese are marked *.

[4] Emil Fischer, *Zeitschr. f. physiol. Chemie*, **33**, 151 [1901]; see also *ibid.* **39**, 155 [1903] and Abderhalden, *ibid.* **44**, 23 [1905], **53**, 19 [1907].

kokoll von einer Verunreinigung des Caseins herrührt." Skraup[1] arrived at a similar conclusion, using sulphuric acid instead of hydrochloric acid. As will be seen from the above data, I have found that the amount of free glycin in ripened cheese is comparatively high; but it must be borne in mind that the glycin may be derived from other proteins and not only from caseinogen. It has already been stated that the cheese-body also contains other proteins such as lactalbumin, lactoglobulin and opalescin; but none of these proteins has yet been investigated, and it is quite possible that they may be found to give glycin.

Though arginin is easily identified by the method described by Kossel and Kutscher[2], and though it has been found to be present in caseinogen, neither Winterstein and his co-workers nor the present writer have succeeded in isolating it from the products formed during the ripening of Emmenthaler and Cheddar cheese. It will be seen from the above data, which are the actual figures found by Emil Fischer[3], that caseinogen contains a moderately large amount of arginin, and I can only explain my inability to find this substance by assuming that arginin is set free from caseinogen in the *early* stages of the ripening—the material used by Winterstein and his co-workers was about six months old—and it is possible that during the course of time it breaks up according to the following reaction:

$$\begin{array}{c}\text{NH}_2\\ \diagup\\ \text{C} = \text{NH}\\ \diagdown\\ \text{NH} . (\text{CH}_2)_3 . \text{CH NH}_2 . \text{COOH} \end{array} \xrightarrow{+\,O_2} \begin{array}{c}\text{NH}_2\\ \diagup\\ \text{C} = \text{NH}\\ \diagdown\\ \text{NH} (\text{CH}_2)_3 . \text{COOH}\end{array} + \text{CO}_2 + \text{NH}_3$$

$$\text{arginin} \qquad\qquad \gamma\text{-guanidinbutyric acid}$$

$$\begin{array}{c}\text{NH}_2\\ \diagup\\ \text{C} = \text{NH}\\ \diagdown\\ \text{NH} (\text{CH}_2)_3 . \text{COOH}\end{array} \xrightarrow{+\,O_2} \begin{array}{c}\text{NH}_2\\ \diagup\\ \text{C} = \text{NH}\\ \diagdown\\ \text{H}_2\end{array} + \begin{array}{c}\text{COOH}\\ \diagup\\ \text{CH}_2\\ | \\ \text{CH}_2\\ \diagdown\\ \text{COOH}\end{array}$$

$$\text{Guanidin} \quad \text{Succinic acid}$$
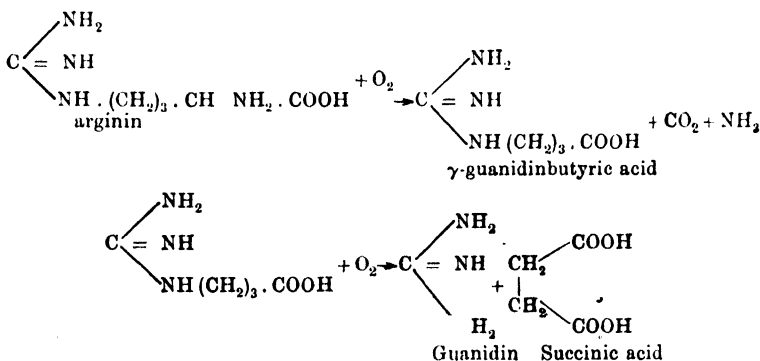
But although a careful search for these products was made I failed to identify them, nor was I able to find any reference to this point by other workers[4].

[1] Skraup, *Monatsheft f. Chemie*, **29**, 791 [1908].

[2] Kossel u. Kutscher, *Zeitschr. f. physiol. Chemie*, **31**, 382 [1900].

[3] Emil Fischer, *ibid.* **33**, 151 [1901].

[4] van Slyke and Hart (*Amer. Chem. Jour.* **29**, 380 [1903]), who have examined an

Several investigators[1] have met with difficulties in their attempts to isolate tyrosin from Emmenthaler cheese. The explanation given by Winterstein and his co-workers is that we are dealing with a transformation of tyrosin into secondary products[2]. However, it must be noticed that none of the secondary products which might be expected have been isolated or identified in cheese. But my figures shew that this is not the case with Cheddar cheese as manufactured in this country. As will be seen, caseinogen contains 4·5 per cent. tyrosin, and the amount I was able to isolate from an old Cheddar cheese about four years old corresponds to 3·8 per cent., which is only 0·7 per cent. less than the amount actually present in caseinogen. An explanation which may be offered is based on the acidity of the curd, which is higher when Cheddar cheese is vatted than is the case with Emmenthaler cheese[3] at this stage of the process of its manufacture. It is possible that the higher acidity would favour the splitting out of tyrosin and perhaps prevent its further decomposition by bacteria.

Previous workers have also failed to obtain glutamic acid in more than traces, though this acid is easily isolated. In this case also Winterstein expresses the view that glutamic acid is used up by bacteria. As will be seen from the data given above, caseinogen contains 11 per cent. glutamic acid, the amount isolated by me is about 7 per cent. It is possible that here also we can trace the explanation of the differences we have observed to the methods of manufacture of the two kinds of cheeses, Emmenthaler and Cheddar respectively.

During these investigations I have been on the look-out for 2 : 5 diketopiperazines[4], which are so frequently met with in the chemistry of proteins and polypeptides. The isolation of these

American Cheddar cheese after 4½ months' ripening, state: "We are led to conclude that arginin does not exist in appreciable quantities in cheese of this age." These authors only obtained from 3 kgrms. cheese, 0·255 gr. arginin.

[1] E. Benecke u. Schulze, *Landwirtsch. Jahrb.* **16**, 317 [1887]; Ulrich Weichmann, *ibid.* 1728 [1888]; Winterstein, *Zeitschr. f. physiol. Chemie*, **41**, 485 [1904]; Bisegger, *Dissertation*, Zurich [1907].

[2] In the semi-popular report by J. A. Coward, "On researches into some of the chemical changes involved in Hard-Cheese Production," p. 16, we find the interesting statement: "Water extracts from cheese (six months old) can readily enough be shewn to contain crystalline bodies and leucin and tyrosin are easily detectable."

[3] M. Nierenstein, *Report on the Swiss Institutions connected with the Milk Industry*, etc. (Chemical Department, University of Bristol, 1909).

[4] See Emil Fischer, *Untersuchungen über Aminosäuren, Polypeptide und Proteine*, p. 80 (Berlin, 1903).

products—the anhydrides of the polypeptides—might very well be of interest in the chemistry of cheese since they are characterised *by the bitterness of their taste,* and it seemed to me quite possible that the "bitterness" of cheese under certain circumstances might be traced to the formation of such products. I have so far failed to isolate any substance of such nature from the material used; I intend, however, to continue my search in this particular direction.

Similar failures were met with during attempts to isolate intermediate polypeptides such as: l-phenylalanyl-d-alanyl-anhydrid or leucylvalyl-anhydrid, found by Abderhalden and Funk[1] in the hydrolytic products of caseinogen. Also here it must be hoped that further work will enlighten this interesting point in the ripening of cheese.

### III. *Secondary products from aminoacids.*

The classical investigations of Czapeck[2] and of Emmerling[3] have shewn that $\alpha$-aminoacids serve as favourable media for the growth of bacteria which transform the $\alpha$-aminoacids into the corresponding aliphatic acids such as acetic, propionic, butyric and other acids. But judging from the presence of aminoacids such as glycin, alanin, leucin, isoleucin, glutamic acid and others—which are all $\alpha$-aminoacids—and the absence (so far as I am able to state at present) of their corresponding aliphatic acids, we must conclude that such processes are of secondary importance in the ripening of cheese and the emphasis laid on this transformation by Bisegger[4] is over-estimated, at least with reference to Cheddar cheese.

Of more interest are the diaminoacids: these yield, after the detachment of the carboxyl-group[5], bases such as: Brieger's diamine, cadaverin, putrescin, phenylethylamin and oxyphenylethylamin, as illustrated in the case of ornithin ($\alpha$-$\delta$-diaminovaleric acid):

$$
\begin{array}{ccc}
CH_2 . NH_2 & & CH_2 . NH_2 \\
| & & | \\
(CH_2)_2 & & (CH_2)_2 \\
| & \rightarrow & | \\
CH . NH_2 & & | \\
| & & CH_2 . NH_2 \\
COOH & & \\
\text{Ornithin} & & \text{Putrescin}
\end{array}
$$

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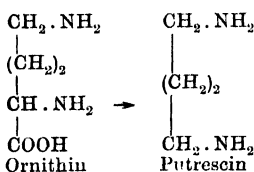
[1] Abderhalden u. Funk, *Zeitschr. f. physiol. Chemie,* **53**, 17 [1907].

[2] Czapeck, *Hofmeister's Beiträge,* **1**, 338 [1902], **2**, 557 [1904].

[3] Emmerling, *Ber. d. deutsch. chem. Gesellsch.* **35**, 2289 [1902].

[4] Bisegger, *Dissertation,* Zurich, pp. 24–27.

[5] Highly interesting are the results of van Slyke and Hart (*Amer. Chem. Jour.* **30**, 1 [1903]) on the relation of carbon dioxide to proteolysis in the ripening of cheese, to which special reference is here made.

Putrescin, phenylethylamin and apparently oxyphenylethylamin have been isolated by Winterstein and Thöni[1] from Emmenthaler cheese. Similar products have also been observed by van Slyke and Hart[2] in American Cheddar cheese. My work has shewn the presence of putrescin, cadaverin and perhaps Brieger's diamine in English Cheddar cheese.

In a previous communication[3] I have shewn that the ethereal extract of an old Cheddar cheese contains an aminovaleric acid which melted at 246—251°. This melting-point did not correspond with any of the known aminovaleric acids and I concluded : "that the product I obtained was probably a mixture of two or three amino-valeric acids." Emil Fischer's[4] investigations of the hydrolysis of caseinogen by acids have shewn that α-aminovaleric acid (M.P. 292—295°) is one of the constituents of these proteins. I have also obtained α-aminovaleric acid on extraction of cheese with alcohol ; this product melted at 287—289° (Slimmer[5] finds for α-aminovaleric acid 291·5°). I hence assume that the aminovaleric acid found by me in the ethereal extract is probably a mixture of α-aminovaleric acid and δ-aminovaleric acid (M.P. 157—158°), the α-aminovaleric acid prevailing. It is possible that the δ-aminovaleric acid is a secondary product formed through putrefaction of ornithin[6].

$$
\begin{array}{ccccc}
CH_2.NH_2 & & CH_2.NH_2 & & CH_2.NH_2 \\
| & & | & & | \\
(CH_2)_2 & & (CH_2)_2 & & (CH_2)_2 \\
| & \rightarrow & | & \rightarrow & | \\
CH.NH_2 & & CH.OH & & CH_2 \\
| & & | & & | \\
COOH & & COOH & & COOH \\
\text{Ornithin} & & \text{α-oxy-δ-aminovaleric acid} & & \text{δ-aminovaleric acid}
\end{array}
$$
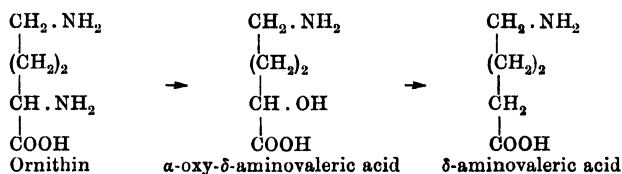
### EXPERIMENTAL.

6874 gr. of Cheddar cheese—about four years old—were extracted with ether in a Soxhlet apparatus, and 5263 gr. of the fat-free product used for the present investigation. This material was extracted in a Soxhlet at first with boiling alcohol, then with a 1 per cent. hydrochloric acid solution in the cold and afterwards with boiling water ; each extraction was examined separately.

[1] Winterstein u. Thöni, *Zeitschr. f. physiol. Chemie*, **36**, 28 [1902].

[2] van Slyke and Hart, *Amer. Chem. Jour.* **29**, 271 [1903].

[3] M. Nierenstein, *Proc. Royal Society*, B. **83**, p. 301 [1911].

[4] Emil Fischer, *Zeitschr. f. physiol. Chemie*, **33**, 151 [1901].

[5] Slimmer, *Ber. d. deutsch. chem. Gesellsch.* **35**, 400 [1902].

[6] Ellinger, *ibid.* **32**, 3542 [1899], *Zeitschr. f. physiol. Chemie*, **53**, 544 [1907]; P. Mayer, *ibid.* **42**, 59 [1904]; E. Baumann, *ibid.* **4**, 312, 417 [1880], *Ber. d. deutsch. chem. Gesellsch.* **12**, 1450 [1878].

The alcoholic extract gave on cooling or on standing over night a white powder which consisted chiefly of caseoglutin.

*Caseoglutin from Cheddar cheese.* The product which had separated out from the alcoholic extract was collected and extracted with warm water (41—45°) so as to remove those aminoacids insoluble in alcohol. This extract, termed "aqueous extract A," was examined separately, and will be dealt with later on. The solid left after extraction with water was once more dissolved in boiling alcohol, and the alcoholic solution, after the caseoglutin had separated out on standing for two days, was filtered and investigated jointly with the main alcoholic extract.

The caseoglutin was washed with hot alcohol and ether and weighed after drying 82 gr. On analysis it gave the following data:

0·1337 gr. gave 17·3 c.c. $N_2$ (20°, 770 m.m.), $N_2 = 14·52 \%$; nitrogen found by other workers[1] for caseoglutin is 15·05 %.

45 gr. were hydrolysed with hydrochloric acid, esterified and distilled *in vacuo* according to Emil Fischer's[2] method. The following fractions were thus obtained:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| I | 8—11 mm. | 27— 45° | 0·6 gr. |
| II | 8—11 mm. | 45— 55° | 4·3 gr. |
| III | 9—14 mm. | 55— 60° | 1·7 gr. |
| IV | 9—15 mm. | 60— 70° | 2·9 gr. |
| V | 9—10 mm. | 70—111° | 4·8 gr. |
| VI | 8—15 mm. | 110—170° | 2·6 gr. |
| Residue | | | 8·7 gr. |
| | | | 25·6 gr. |

The greater part of the glutamic acid was isolated as hydrochloride before the mixture of the aminoacids was esterified. The glutamic acid was then purified and the free acid prepared according to Abderhalden and Rostoski[3]. The acid crystallised from water in long needles, which melted at 194—196°; M.P. for glutamic acid 198°. For further identification the benzoyl-derivative was also prepared, which gave on analysis:

0·1714 gr. gave 0·3608 gr. $CO_2$ and 0·0780 gr. $H_2O$.

$C_{12}H_{13}O_5N$ requires $C = 57·37 \%$ $H = 5·18 \%$.
(Benzoylglutamic acid)
Found $C = 57·41 \%$ $H = 5·06 \%$.

[1] See Abderhalden's *Biochemisches Handlexicon*, **4**, p. 120 [1910].

[2] For details see Emil Fischer, *Über Aminosäuren, Polypeptide und Proteine* [Berlin, 1906] and R. H. A. Plimmer, *The chemical constitution of Proteins*, **1** [London, 1908].

[3] Abderhalden and Rostoski, *Zeitschr. f. physiol. Chemie*, **44**, 265 [1905], **46**, 125 [1905].

A further lot of glutamic acid was obtained from the residue after distillation *in vacuo*. This product melted at 191—195°.

As the aspartic acid ester is generally found in the glutamic ester fraction and crystallises out mixed with the hydrochloride of the latter, I made careful search for this acid but failed to find it.

The fractions obtained on distillation *in vacuo* were examined separately.

### *Fraction I.*

The esters were hydrolysed and the acids isolated according to Emil Fischer. This fraction contained glycin and a small amount of alanin. The latter was identified by means of the benzoyl-derivative. The melting-point was 132—139° (M.P. for benzoyl-d-alanin 147—148°), which is low; however, it must be remembered, as pointed out by Emil Fischer[1], that the benzoyl-derivative of alanin obtained on hydrolysis has often a low M.P. on account of its partial racemisation.

### *Fraction II.*

This fraction contained aminovaleric acid (?) and alanin, the latter predominating. The benzoyl-derivative was prepared, and also here a low melting-point (136--140°) was observed.

On analysis the following data were obtained:

$0.2018$ gr. gave $0.4132$ gr. $CO_2$ and $0.0878$ gr. $H_2O$.

$C_{11}H_{11}NO_3$ requires $C = 55.70\,\%$   $H = 4.64\,\%$.
(Benzoylalanin)

Found   $C = 55.84\,\%$   $H = 4.83\,\%$.

### *Fraction III.*

This fraction contained traces of alanin (benzoyl-derivative, M.P. 134—136°), valin and leucin; however, only alanin was definitely proved to be present.

### *Fraction IV.*

In this fraction I succeeded in definitely identifying both valin and leucin; their isolation was carried out according to Abderhalden[2]. Valin was identified in the form of the benzoyl-derivative, which crystallised in small hairy needles, M.P. 129° (M.P. for benzoylvalin

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[1] Emil Fischer, *Ber. d. deutsch. chem. Gesellsch.* **82**, 2451 [1899].

[2] Abderhalden, *Biochemische Arbeitsmethoden*, **2**, p. 527.

according to Slimmer[1] is 132·5°). Leucin also was identified in form of the benzoyl-derivative, which crystallised from water in small thick prismatic needles, M.P. 100—102° (benzoyl-d-leucin[2], M.P. 104—106°).

## *Fractions IV. and V.*

The main constituent of these two fractions was leucin. The benzoyl- and also the formyl-derivative were prepared. On analysis they gave:

I.   0·2011 gr. gave 0·4910 gr. $CO_2$ and 0·1326 gr. $H_2O$.

$C_{13}H_{12}NO_3$ requires $C = 66·38\,\%$   $H = 7·23\,\%$.
(Benzoyl-leucin)

Found   $C = 66·59\,\%$   $H = 7·32\,\%$.

II.   0·1880 gr. gave 0·3638 gr. $CO_2$ and 0·1405 gr. $H_3O$.

$C_7H_{13}NO_3$ requires $C = 52·77\,\%$   $H = 8·23\,\%$.
(Formyl-leucin)

Found   $C = 52·78\,\%$   $H = 8·36\,\%$.

## *Fraction VI.*

The investigation of this fraction was not successful; however, glutamic acid was found to be present with some certainty.

Similar unsuccessful results were obtained on examining the residue, which apparently contained glutamic acid, arginin and tyrosin.

For the estimation of tyrosin in caseoglutin 15 gr. of the original product were used and the hydrolysis carried out according to the description given by Abderhalden[3]. The tyrosin thus obtained was not pure, but was obtained free of other products when purified according to the methods described by Abderhalden and Teruuchi[4]: the melting-point of the pure product was 302—303°, that of the benzoyl-derivative 176—178°[5].

Lysin and arginin were estimated according to Steudel's[6] method in the remaining 17 gr. The lysin was benzoylated and the dibenzoyl-derivative prepared; the product formed crystals from chloroform and

[1] Slimmer, *Ber. d. deutsch. chem. Gesellsch.* **35**, 400 [1902].

[2] Emil Fischer and Warburg, *ibid.* **38**, 3997 [1905].

[3] Abderhalden, *Handbuch d. biochemischen Arbeitsmethoden*, **2**, p. 493.

[4] Abderhalden u. Teruuchi, *Zeitschr. f. physiol. Chemie*, **45**, 473 [1905].

[5] The M.P. for tyrosin is given by Erlenmeyer und Lippe (*Annal. d. Chemie*, **219**, 170), 290—295°, and by Emil Fischer (*Ber. d. deutsch. chem. Gesellsch.* **32**, 3638 [1900]), 310—314°. The M.P. for the benzoyl-derivative by Erlenmeyer und Harsley (*Annal. d. Chemie*, **307**, 141) 182°, by Emil Fischer (*l.c.*) 191—193°.

[6] Steudel in Abderhalden's *Handbuch der biochemischen Arbeitsmethoden*, **2**, p. 498.

melted at 140—141° (M.P. dibenzoyl-d-lysin 144°, dibenzoyl-d.l.-lysin 145—146°). Arginin was identified in form of the picrate, M.P. 199—200° (M.P. for the arginin-picrate 205—206°).

### ALCOHOLIC EXTRACTS.

The alcoholic extracts (see caseoglutin) were evaporated to $\frac{1}{4}$ of the volume, when a solid crystalline-looking mass separated out. The solid was dissolved in alcohol, and the solution (a) and also the alcoholic filtrate (b) were esterified and distilled *in vacuo*.

*Examination of solution* (a). The following fractions were obtained on distillation:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| I | 12—15 mm. | 30— 45° | 0·8 gr. |
| II | 10—15 mm. | 45— 50° | 6·8 gr. |
| III | 12—15 mm. | 50— 70° | 7·2 gr. |
| IV | 12—15 mm. | 70—115° | 2·4 gr. |
| V | 10—15 mm. | 115—140° | 0·9 gr. |
| Residue | | | 4·2 gr. |
| | | | 22·3 gr. |

### Fraction I.

Gave traces of α-aminovaleric acid. The melting-point of benzoyl-α-aminovaleric acid obtained on benzoylation was 147—149°.

### Fraction II.

Contained α-aminovaleric acid (?), traces of butyric acid and glycin (?).

### Fraction III.

Contained with certainty glycin—the hippuric acid prepared from it melted at 186°—α-aminobutyric acid (?) and valin. The melting-point of the benzoylvalin melted at 131°.

### Fraction IV.

Contained mainly leucin and isoleucin. The separation of these two products was carried out according to F. Ehrlich's[1] method. The benzoyl-derivatives were prepared. The melting-points obtained were, for benzoyl-leucin 102—103° and for benzoyl-isoleucin 114—116°. The latter gave the following data on analysis:

0·2007 gr. gave 0·4863 gr. $CO_2$ and 0·1316 gr. $H_2O$.

$C_{13}H_{12}NO_3$ requires C = 66·38 % H = 7·23 %.

(Benzoyl-isoleucin)

Found C = 67·02 % H = 5·28 %.

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[1] F. Ehrlich, *Ber. d. deutsch. chem. Gesellsch.* **37**, 1828 [1904].

### Fraction V.

This fraction apparently contained leucin (?).

The *residue* consisted of tyrosin and apparently also arginin and lysin. The separation of tyrosin from this mixture was by no means an easy matter, but was eventually carried out successfully. The benzoyl-derivative crystallised from a mixture of benzene and chloroform, melted at 180—181° and gave on analysis the following data:

0·2008 gr. gave 0·4952 gr. $CO_2$ and 0·0959 gr. $H_2O$.

$C_{16}H_5NO_4$ requires C = 67·37 $^o/_o$   H = 5·26 $^o/_o$.
(Benzoyl-tyrosin)

Found    C = 67·25 $^o/_o$   H = 5·30 $^o/_o$.

*Examination of solution* (*b*). The alcoholic extract was saturated with gaseous hydrochloric acid and left standing for 3—4 days in ice, when the hydrochloride of the glutamic acid separated. From this the free acid was prepared, but great difficulties were experienced on attempting to crystallise the product. Finally the raw material was benzoylated, and after several crystallisations from water long needles were obtained, which melted at 112—114°. The benzoyl-derivative was then transformed back into the base according to Emil Fischer's[1] method, and was then found to crystallise from water in platelets which melted at 209—210°. On analysis the following data were obtained:

0·2063 gr. gave 0·3074 gr. $CO_2$ and 0·1129 gr. $H_2O$.

$C_5H_9NO_4$ requires C = 40·82 $^o/_o$   H = 6·12 $^o/_o$.
(Glutamic acid)

Found    C = 40·64 $^o/_o$   H = 6·08 $^o/_o$.

The filtrate from which the above material had been separated was then evaporated and distilled *in vacuo*, when the following fractions were obtained:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| I | 11 rnm. | 30— 35° | 7·5 gr. |
| II | 8 mm. | 55— 66° | 29·8 gr. |
| III | 8 mm. | 66— 82° | 33·6 gr. |
| IV | 8 mm. | 82— 95° | 16·6 gr. |
| V | 9 mm. | 95—115° | 13·5 gr. |
| VI | 8 mm. | 115—140° | 14·5 gr. |
| VII | 6 mm. | 140—160° | 9·9 gr. |
| VIII | 9 mm. | 160—180° | 7·3 gr. |
| Residue | | | 11·4 gr. |

144·1 gr.

[1] Emil Fischer, *Zeitschr. f. physiol. Chemie*, **44**, 265 [1905].

### Fraction I.

This fraction contained mainly α-aminovaleric acid and probably traces of α-aminobutyric acid. The α-aminovaleric acid crystallised from water but did not give a sharp melting-point. The benzoyl-derivative crystallised from water and melted at 145—148°.

On analysis of the α-aminovaleric acid the following data were obtained:

$$0\cdot2731 \text{ gr. gave } 0\cdot5116 \text{ gr. } CO_2 \text{ and } 0\cdot2334 \text{ gr. } H_2O.$$

$$C_5H_{11}O_2N \text{ requires } C = 51\cdot28\,°/_0 \quad H = 9\cdot40\,°/_0.$$

(α-aminovaleric acid)

$$\text{Found} \quad C = 51\cdot09\,°/_0 \quad H = 9\cdot36\,°/_0.$$

### Fraction II.

This fraction, which represented 29·8 gr., was redistilled *in vacuo* and the following three fractions collected:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| a | 12—14 mm. | 20—35° | 0·9 gr. |
| b | 10—15 mm. | 35—40° | 19·7 gr. |
| c | 10—15 mm. | 40—70° | 4·2 gr. |
| Residue | | | 0·6 gr. |
| | | | 25·4 gr. |

The loss of weight was probably due to the fact that fraction II was left standing for some time (from 28th October—22nd November, 1909).

The sub-fractions were examined separately.

### Sub-fraction a.

Contained mainly α-aminovaleric acid. The melting-point of the benzoyl-derivative was 146—148°.

### Sub-fraction b.

As it was expected that this fraction would probably contain mainly glycin, the product was diluted with 300 c.c. absolute alcohol and esterified. To this solution a few decigrams of glycin ester-chloride were added, and left standing in ice for 24 hours. During this time a fair amount of crystals of pure glycin ester-chloride separated out, which gave on treatment with alkali in the usual way and on distillation a liquid which distilled at 47° and 10 mm. pressure. From one part the pure glycin was prepared. It crystallised from absolute alcohol in

small leaflets which melted at 144°, this being the correct melting-point found by Emil Fischer and Skita[1] for glycin. On benzoylation it gave hippuric acid, M.P. 187—188°.

The other half of the ester was transformed into the carboethoxy-derivative and the free carboethoxy-glycin prepared. It crystallised from petrol ether in small prismatic needles, which melted at 72°. On analysis the following data were obtained:

0·2068 gr. gave 0·3087 gr. $CO_2$ and 0·1132 gr. $H_2O$.

$C_5H_9NO_4$ requires C = 40·82 % H = 6·12 %.
(Carboethoxy-glycin)

Found C = 40·71 % H = 6·14 %.

### Sub-fraction c.

This fraction gave traces of glycin (hippuric acid prepared melted at 185—188°) and α-aminobutyric acid (?).

### Fraction III.

This fraction also was once more distilled *in vacuo*, and the following sub-fractions were obtained:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| a | 10—12 mm. | 50—60° | 0·8 gr. |
| b | 10—12 mm. | 60—70° | 1·3 gr. |
| c | 10—12 mm. | 70—75° | 3·2 gr. |
| d | 10—15 mm. | 75—80° | 8·6 gr. |
| e | 10—18 mm. | 80—85° | 0·2 gr. |
| f | 10—18 mm. | 85—90° | 0·4 gr. |
| Residue | | | 0·5 gr. |
| | | | 15·0 gr. |

Also here the loss owing to evaporation was very great and amounted to more than 50 per cent.

Sub-fractions a, b, e and f were not examined.

### Sub-fraction c.

α-aminovaleric acid was obtained with certainty; it formed small prismatic needles from water, which melted at 287—289°. The amount obtained was not enough for analysis. The mother-liquids smelt strongly, suggesting α-oxypyrrolidin-carboxylic acid, and gave the pyrrol reaction. This makes it probable that α-oxypyrrolidin-carboxylic acid is present in cheese: however, it is not certain that this is the case.

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[1] Emil Fischer u. Skita, *Zeitschr. f. physiol. Chemie*, **88**, 177 [1901].

## Sub-fraction d.

All efforts to obtain crystalline products failed, and only different fractions of three different copper-salts were obtained on preparing the salts in aqueous solution and precipitating the salts with absolute alcohol and methylated spirit (F. Ehrlich's[1] method).

Copper-salt I contained 20·60 per cent. Cu, and was probably a mixture of the salts of aminovaleric acid and isoleucin. 0·1004 gr. of the mixture gave 0·0257 gr. CuO.

Copper-salt II contained 20·33 per cent. Cu and was apparently a similar mixture.

Copper-salt III gave on analysis the following data :

0·1049 gr. gave 0·0279 gr. CuO.

0·1104 gr. gave 9·35 c.c. N. 723 mm. 17° C.

Found Cu = 21·2 % and N 9·43 %.

These figures agree more or less for aminovaleric acid, which requires Cu = 21·51 % and N = 12·8 %.

## Fraction IV.

The examination of this fraction was a failure; no crystallisable products were obtained, and once more the copper-salts were prepared. Three salts were obtained and contained from 19—20 per cent. Cu, which indicates that this fraction was a mixture of aminovaleric acid with some other acids of a small carbon content.

## Fraction V.

Contained leucin and apparently isoleucin and α-aminobutyric acid. The leucin was identified on preparing the benzoyl-derivative, which melted at 103—105° and which is the correct melting-point.

This fraction also contained a fair amount of *stearic acid*, which crystallised from alcohol and melted at 65—66°, the correct melting-point for stearic acid being 69—70°. *It is quite possible that the presence of stearic acid in this fraction, and possibly also in the higher-boiling fractions, explains the failure to obtain well crystallised products from the alcoholic extract. It perhaps also explains the failures of other workers in this particular direction.*

## Fractions VI and VII.

These two fractions contained mainly phenylalanin, glutamic acid and traces of aspartic acid (?). The separation of these acids was

[1] F. Ehrlich, *Ber. d. deutsch. chem. Gesellsch.* **37**, 1828 [1904].

carried out according to the method described by Emil Fischer[1]. The phenylalanin crystallised in small plates from water and melted at 278—280°. (The melting-point for phenylalanin is, according to Emil Fischer[2], 283—284°.) When treated with concentrated sulphuric acid and potassium bichromate it gave the characteristic smell of acetaldehyde, probably phenylacetaldehyde[3]. On analysis it gave:

$$0.1811 \text{ gr. gave } 0.4300 \text{ gr. } CO_2 \text{ and } 0.1087 \text{ gr. } H_2O.$$

$C_9H_{11}O_2N$ requires $C = 65.42 \%$ $H = 6.66 \%.$
(Phenylalanin)

Found $C = 64.76 \%$ $H = 6.69 \%.$

The substance was apparently not quite pure.

The glutamic acid was purified in the manner previously described. The free acid crystallised from water and melted at 207—209°.

The residue after the distillation of fraction VII contained phenylalanin, arginin (?) and tyrosin. The phenylalanin crystallised from water and melted at 279—280°. The tyrosin was identified in form of its benzoyl-derivative, which crystallised from benzene and chloroform and melted at 180—181°.

EXAMINATION OF AQUEOUS EXTRACT "A."

This extract, which was obtained on extracting the raw caseoglutin with water, contained mainly glycin and leucin, also traces of α-aminovaleric and α-aminobutyric acids. The aqueous extract was evaporated to dryness *in vacuo* and dissolved in absolute alcohol. The alcoholic solution was then esterified with gaseous hydrochloric acid, when on standing traces of glutamic ester hydrochlorate separated out. On distillation *in vacuo* the following fractions were obtained:

| Fraction | Pressure | Temperature | Quantity |
|---|---|---|---|
| I | 10—12 mm. | 50— 60° | 1·8 gr. |
| II | 8—10 mm. | 60— 70° | 0·7 gr. |
| III | 10—12 mm. | 70— 75° | 0·4 gr. |
| IV | 10—11 mm. | 75—110° | 0·2 gr. |
| Residue | | | 0·3 gr. |
| | | | 3·4 gr. |

*Fraction I.*

Glycin was identified with certainty: traces of α-aminovaleric acid were also present.

[1] Emil Fischer, *Zeitschr. f. physiol. Chemie*, **33**, 174 [1901].
[2] Emil Fischer, *Ber. d. deutsch. chem. Gesellsch.* **33**, 2385 [1906].
[3] Emil Fischer, *Zeitschr. f. physiol. Chemie*, **33**, 174 [1900].

*Fractions II and III.*

Contains α-aminovaleric acid (?), α-aminobutyric and some other acids.

*Fraction IV and Residue.*

Here leucin was identified with certainty, though some other acids were present.

### EXAMINATION OF AQUEOUS EXTRACT.

This part of the investigation was a failure, as scarcely any product was obtained either on extracting the remaining part of the cheese after alcohol extraction with cold water or with dilute hydrochloric acid.

### TYROCASEIN.

25 gr. of the tyrocasein were hydrolysed in the usual manner and only the glutamic acid identified. The free acid melted at 207—209°. For further identification the benzoyl-derivative was also prepared. The latter gave on analysis the following data:

0·1704 gr. gave 0·3584 gr. $CO_2$ and 0·0859 gr. $H_2O$.

$C_{12}H_{13}NO_5$ requires C = 57·37 % H = 5·18 %.
(Benzoylglutamic acid)

Found C = 56·87 % H = 5·03 %.

For the estimation of tyrosin 200 gr. of the tyrocasein were used and the isolation carried out as described previously (see caseoglutin). The melting-point of the pure product was 299—301°, that of the benzoyl-derivative 175—177°.

On analysis of the tyrosin the following data were obtained:

0·2007 gr. gave 0·4378 gr. $CO_2$ and 0·1117 gr. $H_2O$.

$C_9H_{11}NO_3$ requires C = 59·66 % H = 6·07 %.
(Tyrosin)

Found C = 59·49 % H = 6·18 %.

Lysin and arginin were estimated in 200 gr. of tyrocasein. The dibenzoyl-lysin[1] crystallised from chloroform and benzene and melted at 141—143°.

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[1] See Emil Fischer u. Weigert, *Ber. d. deutschen chem. Gesellsch.* **35**, 3772 [1902], also Clara Wildenow, *ibid.* **28**, 3189 [1895] and Drechsel, *Zeitschr. f. physiol. Chemie*, **25**, 527 [1898].

On analysis the following data were obtained:

0·1825 gr. gave 0·4512 gr. $CO_2$ and 0·1031 gr. $H_2O$.

$C_{20}H_{22}O_4N_2$ requires C = 67·80 %. H = 6·22 %.

(Dibenzoyl-lysin)

Found C = 67·51 % H = 6·32 %.

Arginin was identified in the form of its picrate—the melting-point of which was 201—202°, also the nitrate melting at 171°—and the dibenzoyl-derivative—melting-point 211—215°. All these melting-points were found correct.

Histidin was isolated from another 150 gr. according to Steudel[1]. The histidinhydrochloride melted at 227—231°. It gave with bromine-water a red-colouration similar to that described by Knoop[2]. For further identification the anhydride was prepared according to Pauly[3] and also to Emil Fischer and Suzuki[4]. It gave a picrate which melted at 252°, which is in accordance with the observations of the last two authors.

On analysis the following data were obtained:

0·1817 gr. gave 0·3490 gr. $CO_2$ and 0·0862 gr. $H_2O$.

$C_{12}H_{14}N_6O_2$ requires C = 52·55 % H = 5·11 %.

(Histidinanhydrid)

Found C = 52·39 % H = 5·27 %.

For the isolation of the secondary products fresh material was used. 800 gr. of cheese were extracted with ether and from the fat-free material the different products isolated according to Ackermann's[5] method. By this method putrescin and cadaverin were identified[6]. The separation of these two compounds by Ackermann's method is very simple.

The following derivatives of putrescin and cadaverin were prepared:

| | M.P. (Ackermann) | M.P. found |
|---|---|---|
| Putrescin picrate ... ... | 250° | 247—250° (?) |
| „ benzoylate ... | 175—176° | 169—172° |
| Cadaverin picrate ... ... | 221° | Blackens at about this temperature, but did not melt before 237—239° |
| „ dibenzoylate ... | 130° | 130—132° |

[1] Steudel in Abderhalden's *Handbuch d. biochemischen Arbeitsmethoden*, **2**, p. 505.

[2] Knoop, *Beiträge z. chem. Physiol. u. Pathologie*, **11**, 336 [1908].

[3] Pauly, *Zeitschr. f. physiol. Chemie*, **42**, 514 [1904].

[4] Emil Fischer u. Suzuki, *ibid.* **45**, 405 [1905], also *Ber. d. deutsch. chem. Gesellsch.* **38**, 4173 [1905].

[5] Ackermann in Abderhalden's *Handbuch der biochemischen Arbeitsmethoden*, **2**, p. 1002.

[6] Putrescin and cadaverin have also been found in the ethereal extract of Cheddar cheese (M. Nierenstein, *Proc. Roy. Soc. B.* **83**, p. 304 [1911]).

For further identification the pure putrescin-hydrochloride was prepared. It gave on analysis the following data :

> 0·206 gr. gave 0·3654 gr. AgCl.
>
> $C_4H_{12}N_2 + 2HCl$ requires Cl $= 44·06 \%$.
> (Putrescin-hydrochloride)
>
> Found Cl $= 43·76 \%$.

The pure cadaverin-hydrochloride was also analysed and gave on analysis the following data :

> 0·1243 gr. gave 0·1502 gr. AgCl.
>
> $C_5H_{14}N_2 + 2HCl$ requires Cl $= 40·23 \%$.
> (Cadaverin-hydrochloride)
>
> Found Cl $= 39·17 \%$.

Attempts were also made to isolate Brieger's diamine and though a product in small quantity was obtained I failed to identify it with this compound.

# TOXIC EXCRETA OF PLANTS.

BY F. FLETCHER, M.A. (CAMB.), B.SC., &c.

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THE writer has pointed out[1] the influence that some agricultural plants have on others when grown in close proximity to them. It was demonstrated (*inter alia*) that a row of *Sesamum indicum* (gingelly), when sown at a distance of two feet from a row of *Sorghum vulgare* (great millet), will not mature, the plants dying after reaching a height of a few centimetres. These experiments were made at Surat (India) on black cotton soil of a very retentive nature; this character of the soil combined with a rainfall of 42 inches per annum all falling in $3\frac{1}{2}$ months, doubtless emphasized the deleterious effect of the sorghum on the sesamum since the washing of the soil was a minimum.

Russell[2], criticising the experiments on similar lines made by the U.S.A. Department of Agriculture (Bull. 40, Bur. of Soils), objects that

    (i)   seedlings only were used in the work ;

    (ii)  the toxicity in water cultures may be due to bacteria while in the field the result is due to lack of water or food.

The criticisms seemed not to apply to the phenomena previously described by the present writer but it was decided to test the points raised.

With regard to water cultures the writer has already[3] shewn that bacteria do not account for the properties of stale water cultures.

Field experiments were carried out at Gizeh, Egypt, where the rainfall is practically zero and where therefore the water-supply is under complete control. In August, 1909, maize and sesamum were sown in alternate rows, the former crop (like sorghum) being known to be extremely "exhaustive" while sesamum has in previous experiments proved to be extremely sensitive to "exhaustion." The crops were sown as follows:

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[1] Vol. II. No. 8 (Bot. Ser.), *Memoirs of the Department of Agriculture in India*, April, 1908.

[2] *Nature*, August 27, 1908.        [3] *Nature*, June 23, 1910.

Three furrows (1 foot deep) were opened up to take the irrigation water. These are shown in section below:
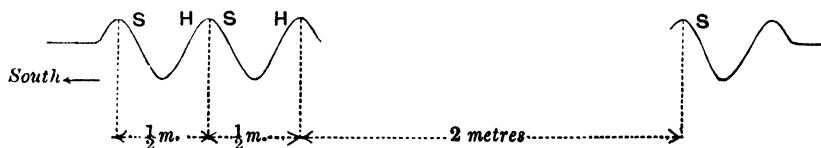


The sesamum and maize were sown in rows along the sides of these furrows at the points marked respectively *S* and *H*.

In plan the rows were therefore as follows:
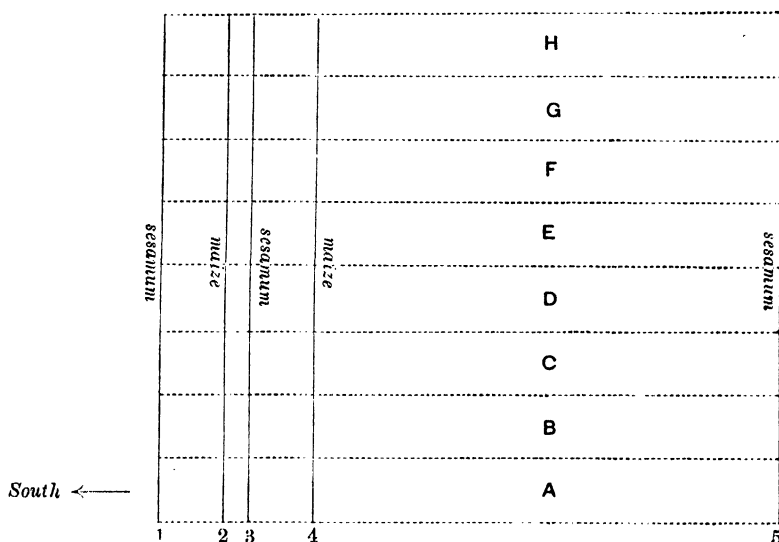


The rows of plants ran east and west so as to obviate the influence of shade as far as possible. Line 1 (sesamum) was not shaded at all until the sun was on the point of setting.

The fifth line (sesamum) was isolated, that is, surrounded by fallow land and therefore uninfluenced by the growth of any other crop.

Each line was divided into eight equal lengths of 25 metres, *A* to *H*. The treatment of these parts in all lines was as follows:

*A* and *E* watered every 15 days; no manure.
*B* and *F*  „  „  10  „  „
*C* and *G*  „  „  2  „  „
*D* and *H*  „  „  2  „; manured every 8 days, each with a dose of $\frac{1}{3}$ kilo sodium nitrate, $\frac{1}{3}$ kilo potassium sulphate, $\frac{1}{3}$ kilo sodium phosphate.

A        B

C        D

Sesamum plants after growing for two months under following conditions :

  (1) between two rows of maize (plant to the left in each group);

  (2) with a row of maize on one side only (middle plant in each group);

  (3) with no maize near (plant to the right).

For treatment of groups (A—D) see text.

In the divisions watered every two days the surface was continuously damp.

Plate IV.[1] shews the average plants of sesamum, two months old, taken from all lines and all divisions, the plant from the third line (between two rows of maize) being always on the left, that from the first line (maize on one side only) in the middle, and that from the fifth line (isolated) to the right.

From the photographs it is obvious that though the extra water and manure have increased the absolute size of the plants grown near the maize, they have not perceptibly increased their size relatively to the size of the plants in the isolated row. In other words, the water and manure have had little or no effect in counteracting the effect of the maize on the neighbouring plants of sesamum, and we conclude therefore that the poor growth of the latter near the former is not due to lack of either water or food-materials.

This experiment corroborates the facts expounded by the writer in the publication above mentioned and appears to prove the excretion of a toxic material by the roots of maize plants.

With regard to the nature of this material, Schreiner and Shorey[2] have isolated dihydroxystearic acid from soils and found it toxic to plants. The writer[3] has by water cultures obtained a solution that behaves like a solution of the sodium salt of dihydroxystearic acid in being salted out by potassium sulphate, etc. The precipitate produced is, however, insoluble in water and the salting out is therefore apparently accompanied by decomposition.

[1] The relative weights have unfortunately been lost. The photographs and weights of the duplicates agree very closely.

[2] *U.S.A. Bur. of Soils*, Bull. 53, April, 1909.

[3] *Cairo Scientific Journal*, **4**, No. 43, April, 1910.

# CARBOHYDRATES OF THE MANGOLD LEAF.

By A. V. CAMPBELL.

(*The Rothamsted Experimental Station.*)

## INTRODUCTION.

THE chemistry of the sugars of foliage leaves and the successive transformation undergone by the various carbohydrates has been the subject of considerable speculation. Reducing substances have been known in foliage leaves for a number of years.

Müller-Thurgau (1), R. Kayser (2) in 1883 were probably the first to discuss the nature of the substances undergoing translocation in green leaves. Sachs (3) concluded that these were derivatives of the starches. A. Meyer (4) in 1885 attempted to ascertain the amounts and nature of these products, and was probably the first to show the existence of true sugars in situ. Brown and Morris in 1893 (*Trans. Chem. Soc.* LXIII. 604) demonstrated the presence of sucrose in foliage leaves and gave strong reasons for regarding it as the immediate product of assimilation and the source of the starch afterwards accumulated in the leaf.

*Experimental.* During the summers of 1909 and 1910 experiments have been carried out in this Laboratory in order to find out if any light might be thrown on these complex metabolic changes which are taking place in green leaves.

Brown and Morris (5) in 1893 attacked the problem, using the leaves of *Helianthus tuberosus* and *Tropaeolum majus*. Their experiments were commenced in order to try and " discover the relation which each sugar bears to the primary assimilation products on the one hand, and to the leaf-starch on the other; to determine, in fact, which are the 'up-grade' sugars towards starch, and to see if any indications were forthcoming of the existence in the leaf of 'down-grade' sugars proceeding from the hydrolysis of starch, a fact of very great importance as bearing upon the physiological mechanism involved in the dissolution of starch in the

living cell. It seemed probable also that the observations on the 'down-grade' sugars might serve to support or not, as the case might be, the supposition of Sachs that the starch is in a continual and rapid state of flux, that, in fact, all the products of assimilation necessarily pass through the form of starch."

The conclusions arrived at by Brown were that :—

(1) Neither dextrose nor levulose were the primary products of assimilation, but rather that cane sugar seemed to be the first sugar synthesised by the assimilatory process.

(2) Cane sugar performed the functions of a temporary reserve material accumulating in the leaf when assimilation is proceeding vigorously.

(3) When the cane sugar reaches a certain amount it is changed into a more permanent reserve material, viz. starch.

(4) The form in which the carbohydrates wander from cell to cell in the leaf are threefold, cane sugar being translocated as dextrose and levulose and starch as maltose.

Although much has been written on the variation in composition and fluctuation of carbohydrates in foliage leaves, no attempts have been made to trace any cycle of events which probably occur in the leaf throughout a period of light and dark, thus leaving us to draw conclusions from a few unconnected determinations. In the hopes of gaining more information as to this cycle the following experiments were carried out.

The work was conducted on the leaves of Mangold (*Beta maritima*). The leaves selected were grown at Rothamsted Experimental Station on a plot giving normal growth. In the preliminary stages a number of difficulties arose. It was found that drying at 100 C. or killing by chloroform ether or toluene before drying, greatly influenced the sugar content.

In the end it was found necessary to kill by immersing the chopped up leaves into boiling alcohol and commencing the extraction at once. Leaves of average size were selected and chopped up after removing the midrib. 50 grs. were taken for the extraction ; the percentage of dry matter being determined in another portion. Extraction of the sugars was effected by boiling with 92 per cent. alcohol, the extraction being continued until all the colouring matter was removed from the leaves. In the preliminary experiments it was found that this was sufficient and that no sugars remained in the leaf-stuff after treatment. The alcohol was then distilled off and the extract finally taken down to

about 20 c.c. on a water bath. A little water was then added and the whole again evaporated until no alcohol remained. The chlorophyll then came out of solution. The solution, after the addition of a little alumina cream or *kieselguhr*, was heated for a few seconds, after which it was made up to 50 c.c. and filtered through a clean dry funnel, the first 40 c.c. being taken for analysis. They were made up to 100 c.c. The cupric reducing power of this extract was then determined, using Pavy's solution. The sugar solution was then inverted by boiling for 7 mins. with 2 per cent. citric acid, and the reducing power again determined. Further hydrolysis was brought about by refluxing a diluted portion with 10 c.c. strong hydrochloric acid for an hour, the acid being then neutralised and a further estimation of the total sugars made. From these determinations it was possible to calculate the percentage of

      (*a*)   Reducing substances (glucose and levulose, &c.).

      (*b*)   Cane sugar.

      (*c*)   Maltose.

These determinations are open to criticism on the ground that they may not be "absolute results." The errors, however, if any, occur in each determination, and will not affect the results for purposes of comparison.

The starch was estimated in the leaf residue by O'Sullivan's diastase method, the conversion being brought about at 62° C. after gelatinisation. Preliminary difficulties having been got over, and a series of duplicate analyses having been done to test the reliability of the methods employed, various types of leaves were examined to contrast colour and also youth and age. One or two determinations were also made to find if the leaves on the several plots showed any marked differences in sugar content owing to the methods of manuring.

Normal leaves were also examined at stated periods to ascertain if there was any difference in sugar content owing to variation of illumination.

Finally an attempt was made to trace the diurnal fluctuations of the sugar.

*Fluctuations.* For this determination leaves were collected every two hours of the day and night during Friday night and Saturday, Sept. 17th, 1910.

Determinations were made in duplicate, the mean results being shown in Table I. and also tabulated in graphical form in Graph I., the percentage of sugars being plotted against the hour of collecting.

TABLE I. *Carbohydrates in Mungold Leaf. Percentages at 2 hour intervals.*

| Time | 6 P.M. | 8 P.M. | 10 P.M. | Midnight | 2 A.M. | 4 A.M. | 6 A.M. | 8 A.M. | 10 A.M. | Midday | 2 P.M. | 4 P.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry matter actual wts. | 4·04 | 3·56 | 3·62 | 3·3 | 3·78 | 3·63 | 3·70 | 4·35 | 4·12 | 4·26 | 4·52 | 4·84 |
| Dextrose and Levulose | 4·67 | 3·58 | 3·98 | 3·92 | 3·81 | 2·64 | 4·70 | 4·43 | 3·44 | 4·33 | 3·57 | 4·65 |
| Cane sugar | 2·20 | 1·46 | 1·23 | 1·47 | ·68 | ·45 | ·79 | ·63 | 1·72 | 1·76 | 1·92 | 2·20 |
| Maltose | ·48 | ·46 | ·79 | 1·57 | 2·23 | 2·40 | 1·37 | 1·32 | ·95 | ·88 | ·97 | ·53 |
| Starch | 8·60 | 8·95 | 9·30 | 9·95 | 7·36 | 6·44 | 5·31 | 4·86 | 5·90 | 6·80 | 8·66 | 9·24 |

TABLE II. *Carbohydrates in Mungold Leaf recalculated as Carbon.*

| Time | 6 P.M. | 8 P.M. | 10 P.M. | Midnight | 2 A.M. | 4 A.M. | 6 A.M. | 8 A.M. | 10 A.M. | Midday | 2 P.M. | 4 P.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dextrose and Levulose | 1·87 | 1·52 | 1·59 | 1·57 | 1·52 | 1·03 | 1·88 | 1·77 | 1·37 | 1·73 | 1·43 | 1·86 |
| Cane sugar | ·92 | ·61 | ·52 | ·62 | ·23 | ·19 | ·33 | ·26 | ·72 | ·74 | ·81 | ·92 |
| Maltose | ·20 | ·19 | ·33 | ·66 | ·93 | 1·01 | ·57 | ·55 | ·39 | ·37 | ·40 | ·22 |
| Starch | 3·82 | 3·98 | 4·14 | 4·41 | 3·26 | 2·86 | 2·36 | 2·16 | 2·62 | 3·02 | 3·85 | 4·11 |
| Total | 6·81 | 6·30 | 6·78 | 7·26 | 5·94 | 5·09 | 5·14 | 4·74 | 5·10 | 5·86 | 6·49 | 7·11 |

GRAPH I.  *Carbohydrates in Mangold Leaf.  Percentages at 2 hour intervals.*
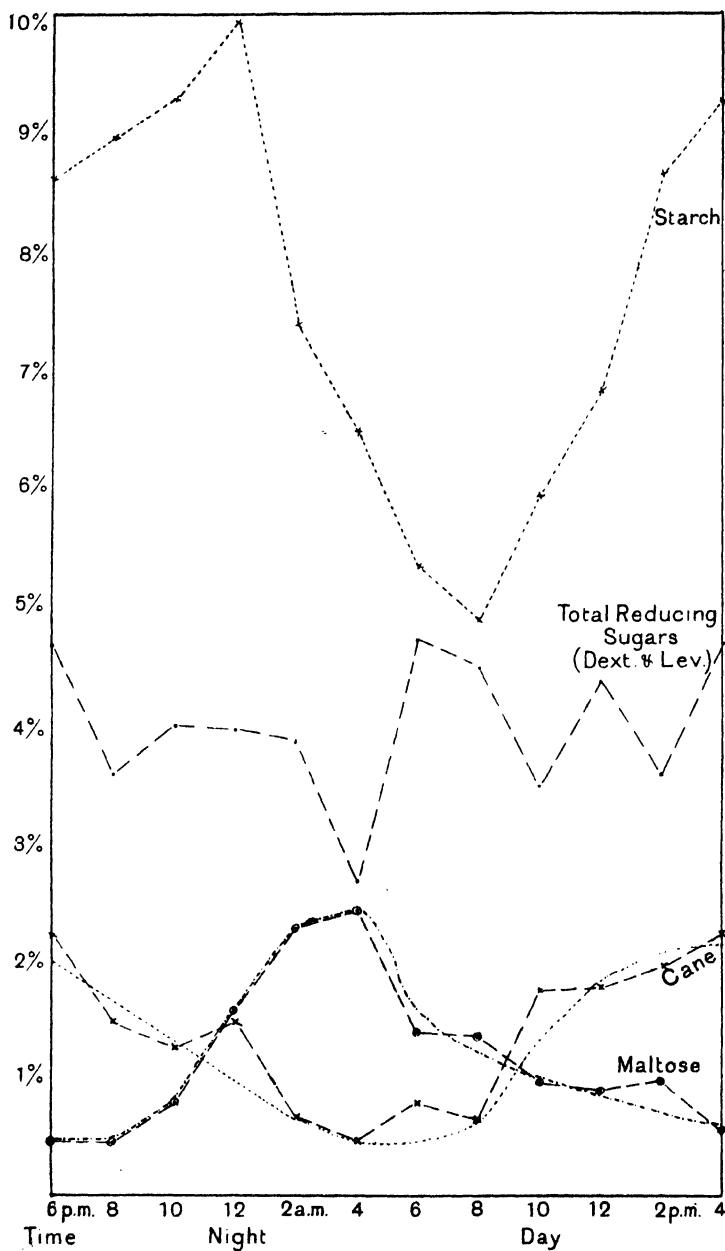
The same determinations calculated in terms of carbon are given in Table II. and Graph II. in order that any conversions may be the more easily noticed.

GRAPH II.  *Carbohydrates in Mangold Leaf calculated as Carbon.*
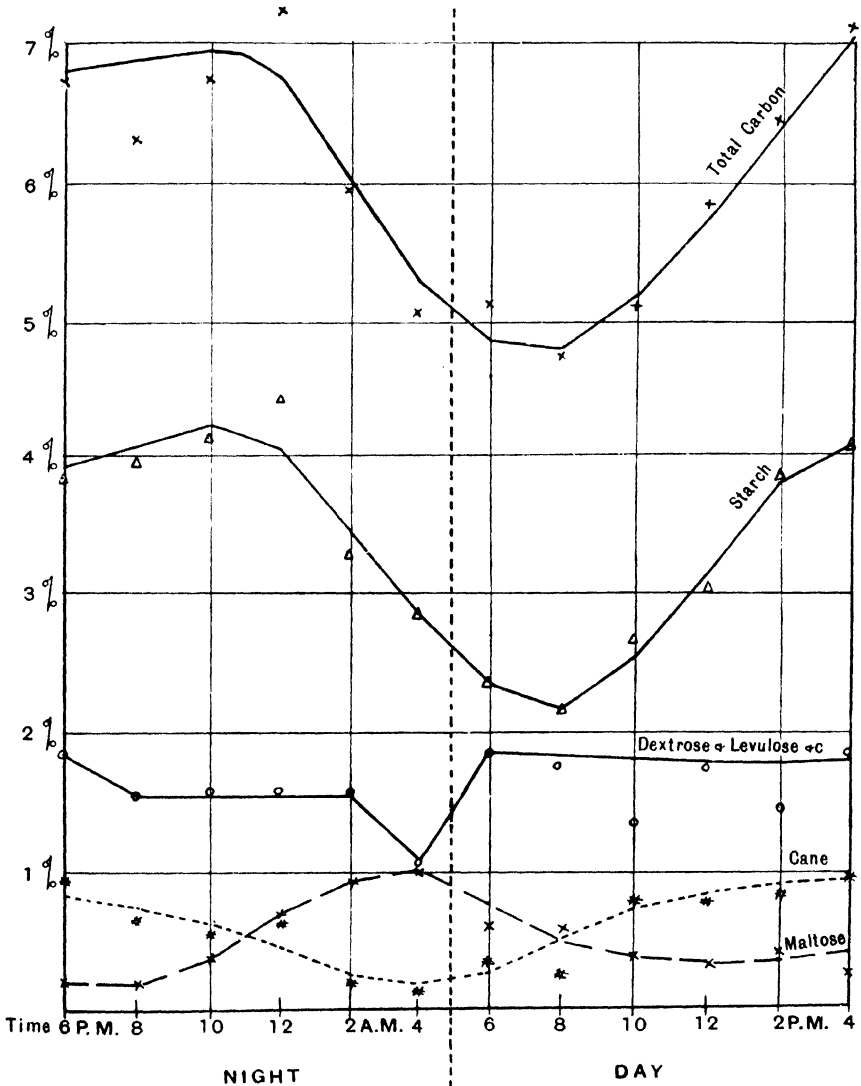
*Discussion of Results.* Before attempting to discuss the significance of the curves it should be noted that the figures are expressed as percentages of the dry matter of the leaf. To follow the actual migration of the carbohydrates it would be necessary to know the absolute weight of a typical leaf, or at least of a unit leaf area. Considerable difficulties are likely to be attached to this determination, which was not carried out in this preliminary work. However, the percentage results will approximate to the truth, since the total weight of the migratory carbohydrates never exceeds 15 per cent. of the dry matter of the leaf and the total fluctuations are within 5 per cent.

*Dextrose and levulose,* together with the other reducing substances, do not appear to fluctuate very much. There is evidence that these reach two levels, the leaf containing an approximately constant percentage in the night-time, and a higher percentage in the day. The fact that the curve jumps up suddenly from 4 to 6 a.m. suggests that the leaf responds very quickly to light. This is also shown by the downward movement from 4 to 6 p.m. Again, this constant low level in the evening when no assimilation is taking place would tend to show that there was not much translocation of dextrose and levulose as such in the night period, or we should expect a gradual depletion of the reducing sugars. It may be, however, that these are formed side by side with maltose by the destruction of starch.

*Cane sugar* varies between ·5 to 2·5 per cent. This is apparently produced in the leaf-matter during periods of illumination. On the days on which the experiment was conducted (Sept. 17) the sun rose at 5.30 a.m., which time synchronises with the beginning of the upward movement of the cane sugar curve. This continues throughout the daytime and begins to drop in the afternoon. This lags behind the glucose curve, beginning its upward movement about an hour later.

In the case of *starch* the fluctuations resemble those of cane sugar, the only differences being (a) the lagging behind (whether this lag is sufficient to justify the hypothesis that starch is formed after cane sugar is a very doubtful point), (b) the continued upward movement from 6 p.m. till 11 p.m. It is suggested that this is due probably to continued elaboration of other carbohydrates, possibly glucose.

*Maltose,* as will be seen from the graphs, fluctuates considerably, but in the opposite sense to the cane sugar, commencing its up-grade movement at 8 p.m. and continuing until the first dawn. This seems to give us an important indication of the sequence of changes in the leaf. The maltose appears to be the last carbohydrate to be formed and

therefore the one most intimately connected with the translocation process. That it is not formed directly may be assumed from the low proportions observed during the light period, unless it is being (a) translocated as fast as formed, which is unlikely, or (b) immediately changed into starch. It is more likely that the maltose is not a direct up-grade product of assimilation, during which period it is also highly probable that little or no translocation is taking place because of the great increase in total carbohydrates.

A few determinations of hemi-cellulose were made in the starch from leaf material by estimating the sugars after hydrolysis for 2½ hours with strong HCl. The results obtained seemed to show that this was probably constant, although they were not at all satisfactory.

The fluctuation of total carbohydrate may be seen from the total carbon curve. That this curve is influenced greatly by sunlight is clearly shown, the upward movement representing a storing up of assimilated products and the downward gradient translocation, though we must bear in mind the fact that these are not absolute results because they are not calculated on to a basis of unit leaf area. Nevertheless we can test their accuracy by comparing the gains and losses with those obtained by other workers. Table III. and Graph III.

TABLE III.

*September 17th, 1910.*

| Time | 6 | 8 | 10 | 12 | 2 | 4 | 6 | 8 | 10 | 12 | 2 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % D.M. | 10·05 | 8·90 | 9·06 | 8·60 | 9·45 | 9·09 | 9·25 | 10·89 | 10·30 | 10·05 | 11·30 | 12·10 |

GRAPH III.
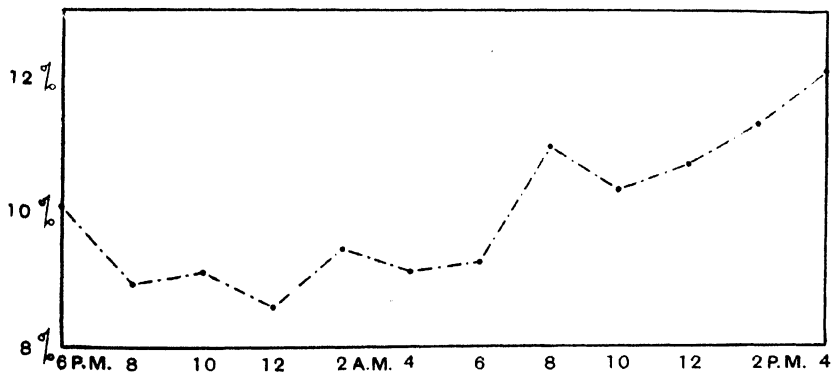
show the percentage of dry matter in mangold leaves taken at the same time as those used for the sugar determinations. The maximum fluctuation in the case of mangold leaves is shown to be about 5 per cent. of the dry weight of the leaf.

That the nett results of assimilation and translocation are of this order may be seen if we compare the results of Brown and Escombe and the later results of Thoday (6). The old method of Sachs is now looked upon as being untrustworthy, since the rates of assimilation measured under his method by direct weighing always give a rate of assimilation about three times greater than that deduced from the intake of $CO_2$. In an actual result by Brown and Escombe, working with *Catalpa bignonioides*, they found:—

1. Increased weight per sq. dec. per hour (Sachs half-leaf method)
$$= \cdot 00669 \text{ grs.}$$
2.     „     „     „     „     „     from $CO_2$ method
$$= \cdot 00235 \text{ grs.}$$

In 1909 Thoday seriously criticised the Sachs method, attributing these higher results to shrinkage, to eliminate which he introduced a stamping method of taking samples. By this method results were obtained which compared favourably with the $CO_2$ intake method. The results were as under:—

*Helianthus tuberosus. Leaves attached to plant. Intermittent sun.*
*Time $7\frac{1}{2}$ hours. 10 A.M. to 5.30 P.M.*

| Leaf | Dry wt. | Gain in dry wt. | Gain $CO_2$ | Gain dry wt. | °/₀ gain dry wt., average |
|------|---------|-----------------|-------------|--------------|---------------------------|
| 1 | ·417 | ·039 | ·061 | ·041 | 9·75 |
| 2 | ·365 | ·023 | ·047 | ·032 | 7·40 |
| 3 | ·393 | − ·006 | − ·007 | − ·006 | — |
| 4 | ·470 | ·024 | ·033 | ·021 | 4·90 |

From this we see that the average percentage increase during seven hours' sunshine was 7·35, which is quite comparable with the results put forward, considering that the day on which the experiment was performed was almost without sun, although being quite bright.

The determinations are neither exact nor numerous enough to enable conclusions to be drawn with any great confidence as to the steps by which assimilation and translocation are effected, but taking the results as they are they seem to suggest that the reducing sugars

are the first carbohydrates to be formed as soon as daylight begins. A little later the cane sugar curve begins to rise, and later still the starch curve. It would also appear that the cane sugar curve does not rise until the reducing sugars have reached the maximum which they maintain throughout the period of illumination. Similarly the starch curve does not rise in its turn until the cane sugar has reached its maximum. It is impossible as yet to lay much stress upon this sequence, but it would seem to suggest that the more elaborate carbohydrate does not begin to form until the simpler one has reached a certain concentration in the cell. The fact that the maltose curve reaches its maximum during the night, and does not rise until the starch curve begins to fall, would indicate that maltose is a down-grade product from the starch, and therefore a form in which the starch is being translocated. If however the carbohydrates leave the leaf in the form of maltose, it is difficult to conceive how this maltose can be transformed into cane sugar, in which form storage in the root takes place.

## VARIATIONS DUE TO MANURING.

The produce of two plots only were analysed in order to find if the sugars in the leaf-stuff varied according to the manures applied. The plots selected were those known as $5\,AC$ and $6\,AC$.

Each of these gets dressings of artificial manures, superphosphate at the rate of 3·5 cwt. per acre and nitrogenous dressings of 2000 lbs. rape cake and 400 lbs. ammonium sulphate per acre. In addition to this $6\,AC$ gets $K_2SO_4$ at the rate of 500 lbs. per acre. The crop on $5\,AC$ is much less than that on $6\,AC$ and is always badly attacked with *Uromyces betae*, the fungus establishing itself early and causing the leaves to become very dark coloured and unhealthy looking. Samples were taken from these plots at 8 a.m. on October 8th. As the results were so very peculiar, the analyses were repeated some days later with the same result.

|  | Oct 8th | | Oct. 14th | |
|---|---|---|---|---|
|  | $6\,AC$, 8 A.M. | $5\,AC$, 8 A.M. | $6\,AC$, 8 A.M. | $5\,AC$, 8 A.M. |
| Dex. and Lev.... | 5·75 | 11 70 | 5·53 | 10·91 |
| Cane sugar ...... | 1·06 | ·35 | 1·13 | ·81 |
| Maltose ......... | 2·86 | 1·78 | 3·65 | 1·94 |

It will be seen from these results that the sugars are for the greater part in the form of glucose and levulose. The meaning of this from the point of view of metabolism is very obscure. Enzymic action and the processes operative in translocation are in some way hindered by the lack of potash, which is probably causing abortive cell tissue which will not allow the free passage of the elaborated carbohydrates. It is suggested, however, that these abnormal conditions that exist are instrumental in providing a " feeding ground" for the fungoid disease with which the plot ,is affected, the attack being due to the abnormal percentage of reducing substances. Previous work by Massee (8) and also by Miyoshi (9) has shown that it is highly probable that this is the case, parasitism being due to the presence of some positively chemiotactic substance within the leaf-stuff, and, moreover, evidence is forthcoming which shows that grape sugar and cane sugar act in a very positive way. Owing to the lateness of the season this work could not be carried further.

### VARIATIONS DUE TO AGE.

A few determinations were made in order to find if there was any difference in the carbohydrate content of young and old leaves. The young leaves seemed to contain rather more dextrose and levulose than the older leaves on the same plant. At the same time determinations were made to see if the carbohydrates were in the same proportion in opposing half leaf samples in order to get some insight into experimental error.

The results were as follows :—

| Date ...... | Sept. 6, 11 A.M. | | Sept. 8, 6 A.M. | | Sept. 8, 7.30 P.M. | | |
|---|---|---|---|---|---|---|---|
| Description ... | Right | Left | Right | Left | Old leaves, Left | Old, Right | Young, Right |
| Dex. and Lev.... | 3·35 | 3·30 | 3·99 | 4·07 | 4·91 | 4·02 | 7·05 |
| Cane sugar ...... | 1·39 | 1·43 | 1·26 | 1·54 | 1·80 | 1·01 | 1·80 |
| Maltose ......... | 1·80 | 1·99 | 1·23 | 2·27 | ·24 | ·24 . | ·66 |

Abnormally dark green leaves were also contrasted with normal leaves, the collections being made on Sept. 6th at 11 a.m. and 5.30 p.m. after a dull day. In this case the maltose in the dark green leaves is rather high. The determination of carbohydrates in the normal leaf at 11 a.m. also shows an exceptionally high percentage of maltose.

Two other determinations were made on similar leaf samples collected at the same time : (*a*) was extracted at once and analysed, (*b*) was killed by boiling in alcohol for a short time, and then allowing to stand for 36 hours in order to see if any hydrolysis was brought about by the acids of the leaf. Apparently no change takes place.

|  | Sept. 6, 11 A.M. | 5.30 P.M., dark green leaf | Sept. 6, 5.15 P.M. | Sept. 6, midnight |  | Aug. 31, 4.30 P.M. | |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | *a* | *b* |
| Dex. and Lev.... | 3·35 | 4·25 | 4·53 | 2·75 | 2·64 | 3·52 | 3·62 |
| Cane sugar ...... | 1·39 | 1·37 | 1·93 | ·49 | ·86 | 2·42 | 2·45 |
| Maltose ......... | 1·80 | 1·34 | ·66 | 1·06 | 1·23 | ·30 | ·01 |

The author is well aware how tentative and preliminary the work described above must be considered. It is only possible to carry it on during a limited period of the year and so far most of the time has been spent in elaborating the method of work, and in making a first series of determinations to ascertain the nature of the problem and order of the quantities to be measured. It is clear that further determinations will have to be made on larger quantities of material and by more refined methods of analysis. Unfortunately the author is precluded from continuing the investigation and therefore desires to put on record the results that have been already obtained.

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# THE AMOUNTS OF NITROGEN AS AMMONIA AND NITRIC (AND NITROUS) ACID IN THE RAIN-WATER COLLECTED AT UITHUIZERMEEDEN, GRONINGEN.

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FOR several years observations have been made on the composition of rain-water, collected by Mr H. Welt, at Uithuizermeeden, a village in the northern part of the Province of Groningen. These observations were made as a part of a research on nitrification in a light clay-soil, by the method of drainage-plots such as are well known from the Rothamsted investigations. The results of these researches appeared in a Dutch journal[1]. In the meantime it seems desirable to publish the results relating to the composition of rain-water.

It may be objected, that, generally, the amount of atmospheric nitrogen precipitated by rain is of no great importance to plant-growth, especially because most of the nitrogen is supplied at times when plants do not need it. It has been shown by Liebscher[2] and other investigators, that for cereals there is one period which is significant for the assimilation of nitrogen. This period may be roughly marked out from about some weeks after the germination to the earing-stage ; it lasts for almost 50 days. So that it will be evident, that from a manuring point of view the atmospheric combined nitrogen does not signify much. After all, the amount is not high, only a few lbs. per acre per annum. But when investigating the nitrogen balance sheet it is quite necessary not to neglect the atmospheric nitrogen compounds. In the determination of the small quantities of nitrates the following method, worked out by Miss Huizinga, was adopted.

[1] *Verslagen van landbouwkundje onderzoekingen der Rijkslandbouwproefstations*, No. x. 911.

[2] *Journ. f. Landw.* 1887, 335.

To about 3 to 5 litres of rain-water, just as much potash solution is added as produces an alkaline reaction, as indicated by phenolphtaleïn. The liquid is boiled in a flask until about 150 c.c. remain[1]. A few c.c. of a decinormal solution of potassium permanganate are carefully added while the liquid is boiling, until the red colour no longer disappears. In this way the nitrites are oxidised. At the same time a slight part of the organic matter, of which the Uithuizermeeden rain-water contains an appreciable amount, is also oxidised, while a thick precipitate of manganese dioxide is formed. This precipitate must be separated from the liquid by filtration. As the organic matter is with difficulty oxidised in alkaline media it is quite necessary to oxidise again, but in an acid medium; sulphuric acid is therefore added to the filtrate which is boiled, then a few c.c. of permanganate solution are carefully added as described above. After this manipulation, potash is added until alkaline, the solution again boiled, then filtered, and the filtrate evaporated in a dish until about 10 c.c. remain. To remove the carbonates 2 c.c. of acetic acid (concentrated) are added, leaving the dish on the waterbath until a few c.c. remain. In this liquid the nitrates are estimated by Schlösing's method.

*Rain-water collected with the pluviometer.*

Rain-water has been collected and analysed since 1900. As, however, the method just described was not employed until July 1908, I only give the results from that date up to the end of 1910; the results from 1900—1908 being not quite exact.

Besides the amounts of nitrogen as ammonia and nitrates, expressed as parts per million, Table I. shows also the relative proportion between these forms of nitrogen. The total amounts of atmospheric nitrogen are very variable, owing chiefly to variations in the amounts of ammonia, the amounts of nitrates being fairly constant.

It may be seen from Fig. 1 that the summits of the ammonia and nitrate curves are coincident in all cases but one, *i.e.* December 1909. It should be important to investigate whether these fluctuations are accidental. The dotted line in Fig. 1 represents rainfall from month to month.

In considering the curves it will become evident that a rise of the nitrogen curves coincides with a fall of the rainfall curve, and

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[1] Sometimes the organic matter separates out in flocks; it must be separated from the liquid by filtering.

inversely; indicating that the concentration of nitrogen is in a certain ratio inversely proportional to the rainfall.

TABLE I.

*Nitrogen as ammonia and nitric acid in rain-water.*

| No. | Date | Nitrogen per million | | | Relative proportions | |
|---|---|---|---|---|---|---|
| | | as ammonia | as nitrates and nitrites | Total | as ammonia | as nitrates and nitrites |
| | **1908** | | | | | |
| 1 | July—August ...... | 0·700 | 0·302 | 1·002 | 69·9 | 30·1 |
| 2 | August—September | ·607 | ·259 | ·866 | 70·0 | 30·0 |
| 3 | Novem.—Decem.... | 1·260 | ·322 | 1·582 | 80·2 | 19·8 |
| | **1909** | | | | | |
| 4 | January—April ... | ·840 | ·239 | 1·079 | 77·9 | 22·1 |
| 5 | May—June ........ | ·980 | ·281 | 1·261 | 77·7 | 22·3 |
| 6 | July ............... | ·607 | ·214 | ·821 | 73·9 | 26·1 |
| 7 | August ............ | ·327 | ·167 | ·494 | 66·2 | 33·8 |
| 8 | September.......... | ·187 | ·167 | ·354 | 52·8 | 47·2 |
| 9 | October ............ | ·840 | ·254 | 1·094 | 76·8 | 23·2 |
| 10 | November .......... | ·653 | ·154 | ·807 | 80·9 | 19·1 |
| 11 | December .......... | ·280 | ·248 | ·528 | 53·0 | 47·0 |
| | **1910** | | | | | |
| 12 | January............. | ·560 | ·207 | ·767 | 73·0 | 27·0 |
| 13 | February—April .. | ·840 | ·224 | 1·064 | 79·0 | 21·0 |
| 14 | May—June ... .... | 1·213 | ·293 | 1·506 | 80·5 | 19·5 |
| 15 | July ............... | ·933 | ·245 | 1·178 | 79·2 | 20·8 |
| 16 | July—August ...... | ·653 | ·217 | ·870 | 75·1 | 24·9 |
| 17 | Septem.—October.. | ·840 | ·242 | 1·082 | 77·6 | 22·4 |
| 18 | Novem.—Decem. ... | ·560 | ·157 | ·717 | 78·1 | 21·9 |

It is greatly to be regretted that the rain-water was not analysed every month. With low rainfalls it was, however, impossible, the quantity of rain-water in the gauge often being insufficient for a trustworthy estimation. Hence the periods of analysing are rather irregularly distributed over the period of observation. This must be taken into account when consulting the figure.

In May, June, July and August 1909, the decrease of ammonia and the increase in the rainfall are rectilineal. In this case the nitrogen concentration seems to be directly proportional to the amount of rain-water. When calculating the amount of ammonia precipitated by rain per month, expressed by the product of parts per million nitrogen and rainfall in mm., it is observed (from Table II.) that these

products are still variable.    This proves that the amount of atmospheric nitrogen brought down by rainfall is not *only* dependent on the amount of rain, but is influenced by other meteorological conditions.
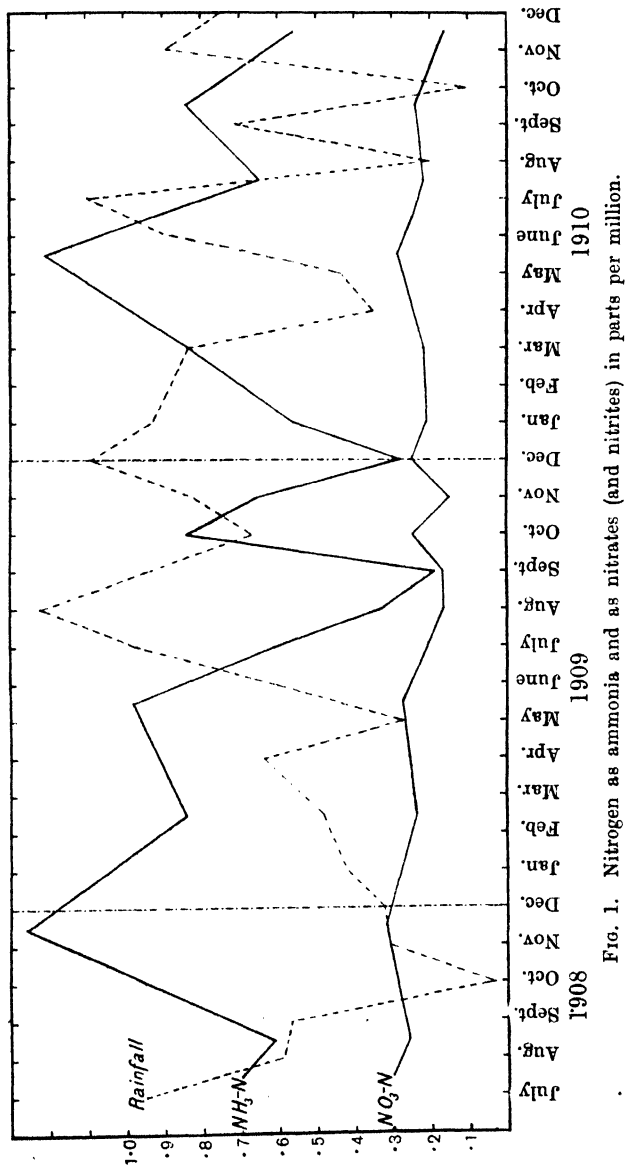


Fig. 1.   Nitrogen as ammonia and as nitrates (and nitrites) in parts per million.

<center>TABLE II.</center>

| 1909 | Rainfall | | Nitrogen per million | | Rainfall × nitrogen (p.p.m.) | |
|---|---|---|---|---|---|---|
| | mm. | inches | as ammonia | as nitrates and nitrites | as ammonia | as nitrates and nitrites |
| January—April | 164 | 6·46 | 0·840 | 0·239 | 137·8 | 39·2 |
| May—June | 88 | 8·47 | ·980 | ·281 | 86·2 | 24·7 |
| July | 97 | 3·82 | ·607 | ·214 | 58·9 | 20·6 |
| August | 123 | 4·85 | ·327 | ·167 | 40·2 | 20·5 |
| September | 94 | 3·70 | ·187 | ·167 | 17·6 | 15·7 |
| October | 67 | 2·64 | ·840 | ·254 | 56·3 | 17·0 |
| November | 82 | 3·23 | ·653 | ·154 | 53·5 | 12·6 |
| December | 109 | 4·29 | ·280 | ·248 | 30·5 | 27·0 |

Whenever the amounts per month are nearly equal, as is the case in July and October 1909, these corresponding results will be due to the laws of chance.

The influence of the amount of the rainfall on the composition of the rain has been in our case very marked.
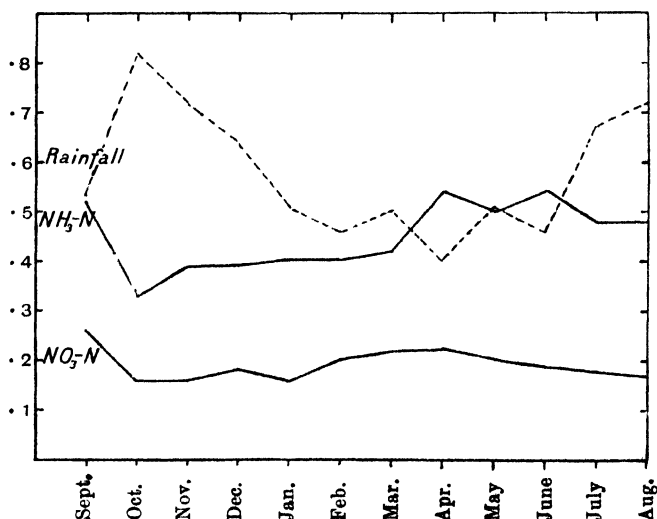


FIG. 2.   Average amounts of nitrogen as ammonia and as nitrates (and nitrites) in parts per million in the rain-water collected at Rothamsted Sept. 1888 to Aug. 1901.   (The rainfall is in mm.)

From the average monthly amounts of nitrogen as ammonia in Rothamsted rain from 1888—9 to 1900—1[1], a figure can be constructed (Fig. 2) in the same way as Fig. 1. This shows an inverse relative proportion exactly as in Fig. 2, but in a less degree.

Miller[2] shows from the Rothamsted data that the concentration with low rainfall is always higher than with high rainfall. With a mean rainfall of 0·65 inch, the rain-water contained 0·965 nitrogen as ammonia and 0·442 nitrogen as nitrates, whilst with a mean rainfall of 4·92 inch, the amounts were 0·278 and 0·124 respectively. These data correspond well with our experience.

It has already been pointed out, that this relation does not exist between the total amounts of nitrogen and rainfall.

The relative proportion between nitrogen as ammonia and nitrogen as nitrates (and nitrites) calculated as an average is

0·724 p.p.m. to 0·237 p.p.m.

75·3 % to 24·7 %.

This proportion corresponds well with the Rothamsted and other data.

*Rain-water collected with the large funnel.*

The rain-water collected with the pluviometer represents a monthly average of individual samples of fluctuating composition. To obtain a better knowledge of these fluctuations I sampled rain-water by means of a funnel of 1 m. diameter, placed in a bottle holding twenty litres. This method enabled us to collect a sufficient sample from a single shower.

The results of the analysis are set out in Table III. with notes on the meteorological conditions.

It will be readily seen from the table, that the composition of the rain is very variable. Fig. 3 shows that the summits of both the curves are not always coincident. Sometimes there is an increase of nitrates and coincidently a decrease of ammonia (1, 2, 3). The relation between the composition and the amount of rain cannot be studied from these data, because the rainfall was measured in only a few cases.

In some cases there seems to be a relation between the composition and the meteorological conditions under which the rain-water was sampled. Thus, for instance, the ammonia percentages were high on

[1] Miller's publication, this *Journal*, I. 283, Table II.

[2] *Ibid.* Table III. p. 284.

TABLE III.

*Nitrogen as ammonia and nitric acid in rain-water collected by the funnel.*

| No. | Days of Sampling | Nitrogen per million | | | Relative proportions | | Conditions of sampling |
|---|---|---|---|---|---|---|---|
| | | as ammonia | as nitrates and nitrites | Total | as ammonia | as nitrates and nitrites | |
| | **1908** | | | | | | |
| 1 | 28 June ............... | 0·843 | 0·364 | 1·207 | 69·8 | 30·2 | N. |
| 2 | 14, 15 July............ | ·655 | ·559 | 1·214 | 53·9 | 46·1 | 3 showers, thunderstorm |
| 3 | 5 August ............ | 1·640 | ·240 | 1·880 | 87·2 | 12·8 | E., thunderstorm, 7 mm. |
| 4 | 11, 12 August......... | ·910 | ·100 | 1·010 | 90·9 | 9·1 | N.W.,  ,,  (hail) |
| 5 | 23 August ...... ..... | 1·027 | ·179 | 1·206 | 85·2 | 14·8 | S.W., shower of 20 mm. |
| 6 | 30, 31 Aug., 1 Sept. | ·467 | ·174 | ·641 | 72·9 | 27·1 | S.W. |
| 7 | 2, 3 September ... | ·467 | ·168 | ·635 | 73·5 | 26·5 | W., thunderstorm, 11 mm. |
| 8 | 9 December ......... | ·513 | ·134 | ·647 | 79·3 | 20·7 | S.S.W., 12 mm. |
| 9 | 10, 11 December ... | ·467 | ·153 | ·620 | 75·3 | 24·7 | S.S.W. |
| | **1909** | | | | | | |
| 10 | 12, 13 April ........ | 1·073 | ·185 | 1·258 | 85·3 | 14·7 | W.N.W. |
| 11 | 18, 25 April ........ | ·513 | ·196 | ·709 | 72·4 | 27·6 | S.S.W. |
| 12 | 17 May ............... | 1·027 | ·224 | 1·251 | 82·1 | 17·9 | N.E. |
| 13 | 23 May, 2 June...... | ·850 | ·224 | 1·074 | 79·1 | 20·9 | S.S.W., thunderstorm |
| 14 | 10 July ............... | ·420 | ·170 | ·590 | 71·1 | 28·9 | S.W. |
| 15 | 28 July ............... | ·373 | ·214 | ·587 | 63·5 | 36·5 | E. |
| 16 | 3 September ...... | ·187 | ·084 | ·271 | 69·0 | 31·0 | W.N.W. |
| 17 | 4 September .... | ·187 | ·125 | ·312 | 59·9 | 40·1 | |
| 18 | 19 September......... | ·653 | ·139 | ·792 | 82·5 | 17·5 | N., 15 mm. |
| 19 | 5, 8 October......... | ·467 | ·130 | ·597 | 78·2 | 21·8 | |
| 20 | 24, 25 October ...... | ·187 | ·147 | ·334 | 56·0 | 44·0 | W. |
| 21 | 27, 28 October ...... | ·467 | ·152 | ·619 | 75·4 | 24·6 | |
| 22 | 26, 30 November ... | ·513 | ·174 | ·687 | 74·7 | 25·3 | |
| 23 | 3 December ......... | ·373 | ·152 | ·525 | 71·1 | 28·9 | S.W., storm |
| 24 | 5, 6 December...... | ·280 | ·174 | ·454 | 61·7 | 38·3 | |
| | **1910** | | | | | | |
| 25 | 12 May ............... | ·747 | ·181 | ·928 | 80·5 | 19·5 | S.E., thunderstorm |
| 26 | 15 May ............... | 1·027 | ·248 | 1·275 | 80·5 | 19·5 | { S.E., thunderstorm,   shower of 14 mm. |
| 27 | 4 June ............... | ·980 | ·238 | 1·218 | 80·5 | 19·5 | Thunderstorm |
| 28 | 8 June ............... | ·467 | ·331 | ·798 | 58·5 | 41·5 | Thunderstorm |
| 29 | 10 June ............... | ·467 | ·243 | ·710 | 65·8 | 34·2 | E.S.E., thunderstorm |
| 30 | 24, 25, 26 June...... | ·373 | ·120 | ·493 | 75·7 | 24·3 | S.W. |
| 31 | 28 June ............... | ·234 | ·103 | ·337 | 69·4 | 30·6 | |
| 32 | 23 July ............... | ·467 | ·082 | ·549 | 85·1 | 14·9 | { W., thunderstorm,   shower of 11 mm. |
| 33 | 23, 25 July............ | ·420 | ·076 | ·496 | 84·7 | 15·3 | S.S.W. |
| 34 | 3, 4 September ... | ·700 | ·130 | ·830 | 84·3 | 15·7 | N.N.W. |
| 35 | 12 September......... | ·800 | ·087 | ·887 | 90·2 | 9·8 | N.E. |
| 36 | 17, 31 October ...... | ·607 | ·248 | ·855 | 71·0 | 29·0 | S.W. |
| 37 | 5 November......... | ·140 | ·078 | ·218 | 64·2 | 35·8 | { W., thunderstorm,   shower of 12 mm. |
| 38 | 6, 9 November...... | ·373 | ·160 | ·533 | 70·0 | 30·0 | S.W., thunderstorm |
| 39 | 9, 10 November ... | ·187 | ·280 | ·467 | 40·0 | 60·0 | W., 10 mm. |
| 40 | 11 November......... | ·233 | ·109 | ·342 | 68·1 | 31·9 | S.E.–S.W., snow |
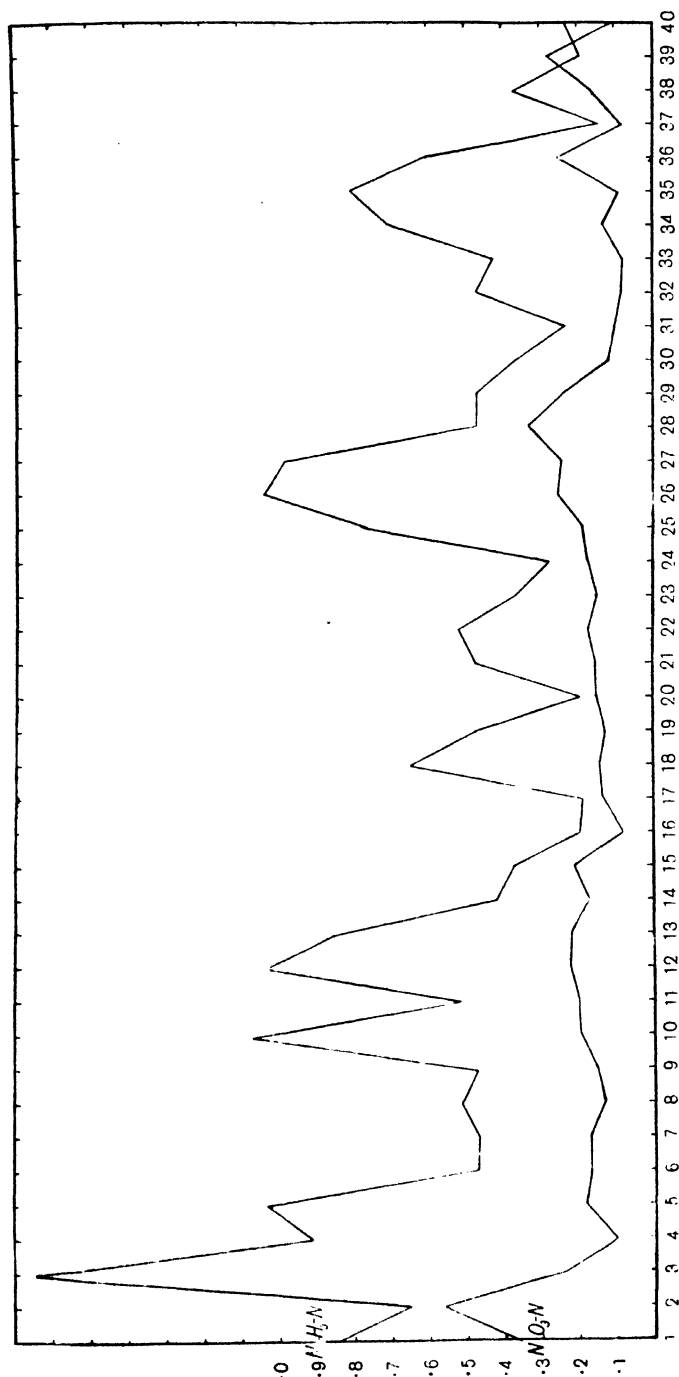
FIG. 3. Amount of nitrogen as ammonia and as nitrates (and nitrites) in parts per million.

5, 11, 12 Aug. 1908, 23 May, 2 June 1909, 15 May, 4 and 8 June 1910 when the samples were collected during thunderstorms. But there are also cases when rain-water was sampled under normal conditions (as, for instance, on 23 Aug. 1908, 12, 13 April, 17 May 1909, 3, 4 and 12 Sept. 1910), when the concentration is not high. Moreover there are many days of thunderstorm when concentration is normal, as for instance 14, 15 July, 2 and 3 Sept. 1908, 12 May, 10 June, 23 July, 5 Nov., 6—9 Nov. 1910.

It must be noticed that when samples are taken for a few days in succession the concentrations do not always differ much:

30, 31 Aug., 1 Sept. and 2, 3 Sept. 1908 (Nos. 6 and 7).

9 Dec. and 10, 11 Dec. 1908 (Nos. 8 and 9).

3 Sept. and 4 Sept. 1909 (Nos. 16 and 17).

3 Dec. and 5 and 6 Dec. 1909 (Nos. 23 and 24).

8 June and 10 June 1910 (Nos. 28 and 29).

23 July and 23—25 July 1910 (Nos. 32 and 33).

It is quite impossible to explain the high concentration sometimes observed, as for instance in the case of a shower of 20 mm. on 23 Aug. 1908 (No. 5). In accordance with the pluviometer-observations already mentioned, one would expect a low percentage of nitrogen in relation to the high rainfall of 20 mm. from one shower without thunderstorm; the nitrogen as ammonia amounted, however, to 1·027 as p.p. millions. A shower of 12 mm. on Dec. 9th (No. 8) had a still lower concentration of 0·513 ammonia as p.p. million.

The relative proportion between ammonia and nitrates calculated as an average has been:

$$0·565 \text{ mgr.} : 0·182 \text{ mgr.}$$

or
$$75·6 °/_0 : 24·4 °/_0.$$

It is noteworthy that this relative proportion corresponds quite exactly with that found by the gauge-observations, although they differ in absolute value.

| | Nitrogen | |
| --- | --- | --- |
| | as ammonia | as nitrates and nitrites |
| Sample from rain-gauge as a mean ... | 0·724 mgr. | 0·237 mgr. |
| „ funnel „ ... | 0·565 ,, | 0·182 ,, |

As the funnel-samples do not represent a mean of rain-water composition, the samples having been taken capriciously, these results do not clash with each other.

For the purpose of studying the relation between the meteorological conditions and the composition of rain-water, researches must be carried out much more systematically.

The mean rainfall in the Netherlands (since 1852) is about 700 mm. Assuming the average composition of the rain-water to be the same as has been calculated from the gauge-observations—·724 mgr. of N as ammonia and ·237 mgr. N as nitrates per litre or 0·961 total nitrogen— the soil only receives 6·73 kg. of nitrogen per hectare per annum.

TABLE IV.

*Rainfall at Uithuizermeeden.*

| | 1901 | 1902 | 1903 | 1904 | 1905 | 1906 | 1907 | 1908 | 1909 | 1910 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mm. | | | | | |
| January ...... | — | 44 | 33 | 35 | 44 | 97 | 23 | 90 | 42 | 93 |
| February...... | — | 19 | 38 | 52 | 32 | 51 | 55 | | 48 | 88 |
| March ........ | — | 42 | 51 | 27 | 60 | 28 | 28 | 50 | | 24 |
| April ......... | — | 48 | 87 | 42 | 60 | 21 | 30 | 28 | 64 | 35 |
| May ............ | — | 67 | 44 | 39 | 15 | 46 | 32 | 64 | 27 | 44 |
| June ........ | 52 | 28 | 73 | 40 | 44 | 48 | 123 | 61 | 61 | 88 |
| July ........... | 60 | 73 | 112 | 57 | 31 | 85 | 41 | 95 | 97 | 110 |
| August ...... | 43 | 136 | 88 | 40 | 70 | 81 | 30 | 59 | 123 | 20 |
| September ... | 29 | 64 | 60 | 13 | 80 | 43 | 31 | 52 | 94 | 71 |
| October ...... | 71 | 36 | 126 | 55 | 148 | 46 | 71 | 3 | 67 | 10 |
| November ... | 98 | 19 | 60 | 85 | 44 | 82 | 36 | 31 | 82 | 89 |
| December ... | 69 | 30 | 25 | 39 | 32 | 43 | 81 | 32 | 109 | 75 |
| Total...... | — | 606 | 797 | 524 | 660 | 671 | 581 | 565 | 814 | 747 |
| | | | | | Inches | | | | | |
| January ...... | — | 1·73 | 1·30 | 1·38 | 1·73 | 3·82 | 0·91 | 3·55 | 1·65 | 3·66 |
| February...... | — | 0·75 | 1·50 | 2·05 | 1·26 | 2·01 | 2·17 | | 1·89 | 3·47 |
| March ......... | — | 1·65 | 2·01 | 1·06 | 2·36 | 1·10 | 1·10 | 1·97 | | 0·95 |
| April ........ | — | 1·89 | 3·43 | 1·65 | 2·36 | 0·83 | 1·18 | 1·10 | 2·52 | 1·38 |
| May ............ | — | 2·64 | 1·73 | 1·54 | 0·59 | 1·81 | 1·26 | 2·52 | 1·06 | 1·73 |
| June ........ | 2·05 | 1·10 | 2·88 | 1·58 | 1·73 | 1·89 | 4·85 | 2 40 | 2·40 | 3·47 |
| July ........... | 2·36 | 2·88 | 4·41 | 2·25 | 1·22 | 3·35 | 1·62 | 3·74 | 3·82 | 4·33 |
| August ...... | 1·69 | 5·36 | 3·47 | 1·58 | 2·76 | 3·19 | 1·18 | 2·32 | 4·85 | 0·79 |
| September ... | 1·14 | 2·52 | 2·36 | 0·51 | 3·15 | 1·69 | 1·22 | 2·05 | 3·70 | 2·80 |
| October ...... | 2·80 | 1·42 | 4 96 | 2·17 | 5·83 | 1·81 | 2·80 | 0·12 | 2·64 | 0·39 |
| November ... | 3·86 | 0·75 | 2·36 | 3·35 | 1·73 | 3·23 | 1·42 | 1·22 | 3·23 | 3·51 |
| December ... | 2·72 | 1·18 | 0·99 | 1·54 | 1·26 | 1·69 | 3·19 | 1·26 | 4·29 | 2·96 |
| Total...... | — | 23·87 | 31·40 | 20·66 | 25·98 | 26·45 | 22·91 | 22·25 | 32·05 | 29·44 |

# A NOTE ON ONION COUCH.

By L. M. UNDERWOOD, B.Sc.

(*The Rothamsted Experimental Station.*)

ONE of the commonest grasses found in hedges and thickets in this country, more especially on the lighter soils, is the Tall Oat Grass (*Arrhenatherum avenaceum*, Beauvais). In meadows it is generally present, abundantly on some soils, while occasionally it forms part of the ' seeds " mixture put down for temporary or permanent pasture. In some localities a form of this grass known as " Onion Couch " becomes a dangerous weed on the arable land—this plant is identical in outward appearance with the Tall Oat Grass except at the base of the stems where the nodes swell and form chains of bulbs each of which is capable of breaking off and giving rise to a new plant. This habit of growth together with a liberal supply of seeds formed in June and July make it a very difficult weed to eradicate. Its occurrence is usually reported from the lighter soils, but in the West of England the heavy land is equally badly affected.

Botanists differ as to whether the bulbous form is entitled to specific rank. Bentham and Hooker do not recognise the onion form at all while Reichenbach distinguishes it as *Arrhenatherum nodosum* and Lindley *Arrhenatherum bulbosum*. The question is whether the bulb structure is a congenital variation of specific value or the outcome of physiological response to the conditions of the habitat, such as for example, arable land in distinction to meadow, shading, wet or dry soil, heavy or light land. To test this point the following experiments were arranged.

Seed of the onion couch was procured from Perse Condle, Dorset, where the plant abounds on a heavy soil derived from the Oolite, and some Tall Oat Grass seed was collected at Rothamsted. No difference could be detected between the two samples. Glazed earthenware pots

1 and 2.  Rothamsted Soil.  25 % water.
  1.  Seeds of Tall Oat Grass.
  2.        ,,         ,,        Onion form.



3 and 4.  Rothamsted Soil.  30 % water.
  3.  Seeds of Tall Oat Grass.
  4.        ,,         ,,        Onion form.

of about 14 cm. in diameter and 18 cm. high were prepared to afford the following comparisons.

1. *Control.*　Rothamsted soil $+ 10\,\%$ coarse sand to improve the texture.　Moisture content 25 %.
2. *Wet Soil.*　Moisture content increased to 30 %.
3. *Dry Soil.*　Moisture content decreased to 20 %.
4. *Shading.*　Round the rim of the pots containing soil as in the control, cylinders of brown paper were placed, so as to rise eight inches above the surface.
5. *Evaporation Checked.*　Round the rim of the pots glass cylinders (eight inches high) were placed.
6. *Thicker Seeding.*
7. *Sandy Soil.*　A very light sandy soil used.　Moisture content 15 %.
8. *Clay Soil.*　A mixture of heavy clays used.　Moisture content 25 %.

In each case four pots were prepared, two were sown with seed from the ordinary Oat Grass and two with seed of the bulbous form. The pots were watered once a week to keep the moisture constant. The seed was sown in September 1910, the plants germinated and were well up before the winter set in but no differences were noticeable. Bulbs began to appear in March 1911 and by May all the pots sown with seed from the onion couch were producing chains of bulbs while the Tall Oat Grass pots remained normal. This shows that the habit of forming bulbs is hereditary and does not depend on the conditions of the habitat. Other than the bulbs, no differences in habit were noted between the two plants.

The experiment was suggested by and carried out under the direction of Mr A. D. Hall, F.R.S., to whom my best thanks are due.

# COPPER FUNGICIDES.

## By SPENCER UMFREVILLE PICKERING, M.A., F.R.S.

INVESTIGATIONS on the various products of the action of lime on copper sulphate[1], including ordinary Bordeaux mixture, led to the conclusion that the efficacy of such substances as fungicides depended on the proportion of copper in them which is rendered soluble by the carbon dioxide of the air, and, that if a deficiency of lime is used to start with, one of the lower basic sulphates of copper is obtained, from which carbon dioxide liberates a much larger proportion of copper than it does from the more highly basic sulphates present in ordinary Bordeaux mixture. The lowest basic sulphate is, owing to its physical condition, unsuitable for spraying purposes; but this is not so with the compound $10\,CuO, SO_3$, obtained by adding lime-water to copper sulphate just short of alkalinity, and this substance has been put on the market under the name of Woburn Bordeaux Paste. In the absence of secondary reactions, carbon dioxide should convert one-tenth of the copper present in it into soluble copper sulphate, $2\,(10\,CuO, SO_3) + 9\,CO_2 = 9\,(2\,CuO, CO_2) + 2\,CuSO_4$, whereas with ordinary Bordeaux mixture, any copper sulphate formed in this way, would be decomposed by the excess of lime present, and that ultimately remaining undecomposed would be very small in amount. Direct experiments led to the conclusion that under the action of carbon dioxide the proportion of copper rendered soluble from Woburn Bordeaux was 18 to 20 times greater than that from ordinary Bordeaux, and this ratio was found to agree with the relative energy with which the two substances scorched foliage, as well as with their behaviour in other respects. At the same time it was pointed out that under the conditions obtaining in practical spraying, such a proportion might not obtain, and, pending further

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[1] *Eleventh Report of the Woburn Experimental Fruit Farm*, 1910; this *Journal*, III. 171; *Trans. Chem. Soc.* 1907, 1908, and 1909, 1411.

direct trials, it was recommended that the proportion of Woburn Bordeaux should not be reduced below one-twelfth of that of ordinary Bordeaux. Recent experiments have indicated that even this proportion is too small, one-sixth being, apparently, the equivalent proportion, *i.e.* ordinary Bordeaux is equivalent in its fungicidal action to Woburn Bordeaux containing only one-sixth as much copper.

The evidence on which this proportion is based will not be discussed in the present communication, but only the principles underlying the difference in potency of the two substances.

Gimingham and Barker[1] question the validity of the principle that solubility of the copper compound is essential in order that it may exert its fungicidal action, and doubt that such solubility is brought about by the action of carbon dioxide, suggesting that mere contact between the fungus and an insoluble copper compound is sufficient to account for fungicidal action. They show, in the first place, that if air, instead of carbon dioxide, is passed through a liquid containing the basic sulphates, only a very small, instead of a large, proportion of the copper becomes soluble, and that if the comparatively strong copper solution obtained by the action of carbon dioxide is exposed to air, nearly all of the copper becomes deposited[2]. This latter fact was repeatedly noticed by the present writer, and it was certainly an omission on his part that it did not lead him to differentiate between the action of carbon dioxide and of ordinary air on these compounds. But it is questionable how far such a differentiation is of importance from the point of view of practical spraying, for any fluid sprayed on to the leaves of trees will soon find itself bathed in an atmosphere of the carbon dioxide evolved from the leaves themselves.

Gimingham further questions whether the carbon dioxide in air can render soluble any, or more than mere traces of the copper in ordinary Bordeaux mixture. He seems, however, to have overlooked a very simple experiment which places this point beyond dispute, namely, that when a drop of Bordeaux mixture is placed on a piece of paper impregnated with potassium ferrocyanide, as soon as it dries up, and communication between the excess of lime and the particles of basic

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[1] This *Journal*, IV. 69 and 76.

[2] Throughout his paper, Gimingham speaks of the amount of copper remaining in solution in this and other similar cases, as *nil*. This simply means that it was less than that recognisable by the ordinary ferrocyanide test on which he relied. As will be shown below, the action of the liquids on iron would have proved that there was copper still in solution; and the ferrocyanide test would have done the same, if the liquids had been concentrated by evaporation.

sulphate is thereby prevented, the red colour of copper ferrocyanide makes its appearance.

This experiment may be modified so as to give simple and incontrovertible evidence that it is the carbon dioxide in the air which renders the copper in Bordeaux mixture soluble. Into two similar tubes, pieces of ferrocyanide paper with a drop of Bordeaux mixture on them are introduced: a current of air is then drawn through the tubes, this air being deprived of its carbon dioxide before entering the second one: the paper in this tube remains unaltered in colour, whilst that in the first tube becomes red in a few minutes.

It may also be added that the invariable presence of dissolved copper in the drip-water collected from trees sprayed with Bordeaux mixture has been fully established by Crandall[1], though in this case solvent agencies other than the carbon dioxide of the air were not excluded.

No doubt, the actual copper contents of any solution obtained by the action of air on Bordeaux mixture may be very small, but Barker and Gimingham do not seem to have realised the extent to which even such very dilute solutions may operate, when brought into contact with any substance which can utilise and remove the copper as soon as it passes into solution. Some of the simple experiments quoted below will give striking illustrations of this.

In the second paper alluded to, Barker and Gimingham seek to prove that the solvent of normally insoluble copper compound is a substance excreted by the fungus itself. That fungus spores while germinating, and during growth, excrete a substance capable of acting on the cell walls of the host plant, cannot be doubted, and that this substance may be capable of dissolving copper compounds was freely admitted (*Eleventh Woburn Report*, p. 107); but it seems impossible to attribute any such excretive action to dormant spores, without applying a novel meaning to the word "dormant." Whether, however, it is dormant or active spores which are in question, the excretion of a solvent substance from them could only be established by examining their action on a copper compound which is really insoluble in water, and this Barker and Gimingham have not done; for all their experiments have been performed with 10 CuO, SO₃, which is not insoluble. As clearly indicated by the present writer, although this compound does not dissolve sufficiently to give a reaction with ferrocyanide by testing

[1] Bulletin No. 135 of the University of Illinois Agricultural Experiment Station.

in the ordinary way, it does dissolve sufficiently to react with iron, and this reaction soon becomes very considerable: in fact, so far are Barker and Gimingham's experiments from establishing the excretion of solvent matter from fungus spores, that they would have obtained exactly the same, or strictly similar, results, had they substituted a piece of metallic iron for the spores in question.

In the following experiments a current of air or other gas was passed at the rate of 8 litres an hour through 225 c.c. of liquid containing 0·375 grams of copper in the form of one of the basic sulphates, and in the liquid were placed two bright iron rods, weighing together about 30 grams, and presenting a surface of 28 sq. cm. to the liquid. These were removed at intervals, and weighed, any deposit of copper on them being rubbed off with a cloth. The loss in weight of the rods gave, of course, the amount of copper abstracted from the liquid. In duplicate experiments this loss was fairly constant[1], but not sufficiently so to be trustworthy; consequently, two determinations, and only two, were always made at the same time in the same current of gas, one of these being used as the standard of comparison, and this was always one in which the copper was present in the form of 10 CuO, SO₃, made by adding lime-water to the normal sulphate just short of alkalinity.

In illustration of the character of the action the following results with 10 CuO, SO₃ may be quoted, the apparent loss in weight of the iron being translated into its equivalent of copper, and expressed as a percentage of the total copper originally suspended as basic sulphate in the liquid.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1. | After 10 hours | ...... | 10·9 | per cent. | copper removed | | | |
| 2. | After 10 hours | more | 7·0 | ,, | more | copper | removed | |
| 3. | ,, | ,, | ,, | 6·4 | ,, | ,, | ,, | ,, |
| 4. | ,, | ,, | ,, | 3·6 | ,, | ,, | ,, | ,, |
| 5. | ,, | ,, | ,, | 2·0 | ,, | ,, | ,, | ,, |
| 6. | ,, | ,, | ,, | 1·7 | ,, | ,, | ,, | ,, |
| 7. | ,, | ,, | ,, | 7·5 | ,, | ,, | ,, | ,, |
| 8. | ,, | ,, | ,, | 4·3 | ,, | ,, | ,, | ,, |
| 9. | ,, | ,, | ,, | 8·6 | ,, | ,, | ,, | ,, |
| 10. | ,, | ,, | ,, | 7·5 | ,, | ,, | ,, | ,, |
| 11. | ,, | ,, | ,, | 4·5 | ,, | ,, | ,, | ,, |
| 12. | ,, | ,, | ,, | 2·6 | ,, | ,, | ,, | ,, |

Thus, during the first 10 hours, 10·9 per cent. of the copper was removed; but the rate of removal decreased rapidly down to 1·7 per

[1] In seven cases the copper equivalent of the iron dissolved in 3 hrs. varied from 3·5 to 4·7 per cent., with a mean of 4·2,

cent. per 10 hours after 50 hours had elapsed; this decrease, however, is mainly due to the iron becoming covered with an irremovable coating of copper or iron oxide, for on substituting fresh bright pieces of iron for the first ones, as was done after the 6th and 8th observations, the original rapid rate of action was nearly restored. If the iron could be kept permanently bright, this rate of action would be maintained, and, indeed, would be greater than any of the values entered above; for during the first hour alone it amounted to 1·5 per cent. At that rate it would require only 70 hours to remove the whole of the copper present: and this is the case, be it noted, with a substance which dissolves to a smaller extent than ·00005 gram of copper in 100 c.c.

The loss in weight of the iron, however, in these experiments may give a somewhat exaggerated estimate of the amount of copper removed, at any rate, when the action has proceeded for some time, since a secondary reaction occurs, as previously mentioned, the metallic copper and iron forming a couple which electrolyses the liquid, producing hydrogen and iron oxide.

The relative action of air on the various copper compounds is sufficiently illustrated by the following values obtained after three hours, though confirmatory results were also obtained after both shorter and longer intervals.

|  | $2 \cdot 5 (4 CuO, SO_3)$ | $10 CuO, SO_3$ | $10 CuO, SO_3, 3 CaO$ | $5 (2 CuO, CO_2)$ |
|---|---|---|---|---|
| Per cent. copper removed | 7·3 | 4·2 | 2·3 | 1·0 |
| Relative values ............ | 174 | 100 | 54 | 23 |

The sulphate, $4 CuO, SO_3$, is the least basic one obtained by the action of lime on copper sulphate, and is more soluble than $10 CuO, SO_3$ whilst $10 CuO, SO_3, 3 CaO$, is the most basic one, and is that present, together with excess of lime, in ordinary Bordeaux mixture; it is strongly alkaline, and insoluble, and does not itself act on iron; but as soon as carbon dioxide is passed into the liquid containing it, it begins to be decomposed with the formation of $10 CuO, SO_3$, and the mixture, though still alkaline, acts on the iron at the rate indicated. Ordinary copper carbonate, as will be seen, exercises only one-quarter as energetic an action as the sulphate $10 CuO, SO_3$ (quantities containing equal weights of copper being compared), and in some cases the action with the carbonate was found to be much more feeble than that in the experiment here recorded.

When ordinary Bordeaux mixture itself was examined in this way, taking a mixture made from equal weights of crystallized sulphate and

calcium oxide, the iron remained perfectly bright in it till the current of air had been passed through it for 130 hours; then, all the free lime having been carbonated[1], the basic sulphate began to be attacked, and the iron was soon acted upon at the rate indicated above for 10 CuO, SO$_3$, 3 CaO.

A simple experiment of this sort must place it beyond doubt that the copper in Bordeaux mixture is brought into the soluble condition by the action of air, and the greater the excess of lime present, the longer is the interval before this action commences, though in practical spraying it will always be much shorter than in this experiment, owing to the deposit drying up and isolating the particles of basic sulphate from those of lime.

The following experiments with Woburn Bordeaux (10 CuO, SO$_3$), confirm the writer's previous results as to the great increase of the action produced by an atmosphere of carbon dioxide, but they show, at the same time, that the action is actually reduced by carbon dioxide until this latter reaches a certain proportion. Except in the last experiment, the results were obtained by drawing the air charged with carbon dioxide, first through a bottle containing the basic sulphate, next through apparatus capable of absorbing this gas, and then through a second bottle with basic sulphate. In the last experiment, where carbon dioxide itself was used, the two bottles had to be separate, the rates of it, in the one case, and of the air, in the other, being adjusted so as to be as nearly the same as possible. In the third experiment the proportion of carbon dioxide was not determined, the air being drawn from a funnel suspended over a burning candle. The results refer to observations made after 3 hours' passage of the gas.

|  | Air containing no CO$_2$ | Air with ·03 per cent. CO$_2$ | Air with $x$ per cent. CO$_2$ | Air with 10 per cent. CO$_2$ | CO$_2$ only |
|---|---|---|---|---|---|
| Relative action... | 109 | 100 | 38 | 58 | 834 |

Thus by depriving the air of its carbon dioxide the action is increased by 9 per cent., and by increasing the proportion of carbon dioxide to a certain extent the action is reduced, though what the minimum point is, and what proportion of carbon dioxide produces it, has not been determined. A further increase in the proportion of the gas then increases the reaction till, with the undiluted gas, the action is more than eight times as rapid as with ordinary air.

[1] The total air used contained about twice as much carbon dioxide as that required by the lime, but the current was much too rapid for it all to be utilised.

As bearing on the question of the nature of the chemical reaction occurring in this case, the influence of the presence of calcium sulphate on the amount of copper rendered soluble by carbon dioxide must be borne in mind. According to the equation given above this gas should render soluble only 10 per cent. of the total copper present, but experiments generally indicated as much as 50 per cent. passing into solution (*Eleventh Woburn Report*, pp. 31, 63), and this was traced mainly to the presence of the calcium sulphate resulting from the action of the lime on the copper sulphate originally taken. The influence of this calcium sulphate is further illustrated by the following experiments with iron, where in the case of A the precipitate of basic sulphate was washed free from admixed calcium sulphate[1], in that of B it was not so washed, and in that of C an extra equivalent of calcium sulphate was added.

| | A, No $CaSO_4$ | B, $9\,CaSO_4$ to each $10\,Cu$ | C, $18\,CaSO_4$ to each $10\,Cu$ |
|---|---|---|---|
| Relative action, 3 hours...... | 79 | 100 | 121 |

A similar experiment with copper carbonate showed that in that case the presence of calcium sulphate had a still greater effect, increasing the action six-fold (D and E, below), and rendering such a mixture three-quarters as effective as Woburn Bordeaux itself[2] (B); whereas calcium chloride (F) only doubled the action, and calcium carbonate (G) produced no effect at all.

| | $10\,CuO, SO_3$ B, | Nil D, | $5\,(CuCO_3, CuO)$ with | | |
|---|---|---|---|---|---|
| | | | $20\,CaSO_4$ E, | $20\,CaCl_2$ F, | $20\,CaCO_3$ G, |
| Relative action... | 100 | 12 | 76 | 28 | 12 |

An exact analysis of the chemical reactions involved in the above results would probably be a tedious matter, but they do not appear to present any fundamental difficulties. The basic sulphate $10\,CuO, SO_3$ is itself soluble in water, and, hence, acts on iron: the first action of carbon dioxide upon it is probably to produce a compound composed of a basic sulphate with basic carbonate, similar to that obtained by precipitating copper sulphate with acid sodium carbonate (*Chem. Soc. Trans.* 1909, 1415), and if this is less soluble than the original basic sulphate, the reduction in the action of iron would be accounted for.

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[1] Some calcium sulphate is always retained by the basic sulphate in a state of combination (*loc. cit.* p. 49).

[2] It would, of course, owing to its physical condition, be much less effective as a spray fluid in practice.

A further increase in the proportion of carbon dioxide would convert the basic into the normal carbonate, and this would probably form with copper sulphate a cupricompound, exhibiting, as such cupricompounds do, a relatively great solubility, which would account for the great increase in activity as regards iron. Many cupricarbonates have been obtained (*loc. cit.* 1911, 99, 800) which may be regarded as containing the elements of $CuCO_3$ with those of some other carbonate, and, as was pointed out at the time, a sulphate or other salt might probably be substituted for one or other of the carbonates: since then several substances of that sort have been obtained. The formation of these will also explain the increased activity produced by the addition of calcium sulphate, either to the basic sulphate or carbonate of copper.

The values given on p. 277 show that in spite of the small solubility of $10\,CuO$, $SO_3$ the greater part of the copper in it may pass into solution and be utilised within a comparatively short time, provided there is any body present, such as iron, to react with it and remove it from the solution. This will occur equally if it be removed by absorption into a fungus spore or the leaf of a plant, and the absorption in that case should be at a much greater rate than in these experiments, because the reacting surface exposed to a given amount of copper would be far greater. The relative action with 1, 2 and 10 iron rods in liquids containing the same amount of basic sulphate was found to be

|                | 1 rod | 2 rods | 10 rods |
|----------------|-------|--------|---------|
| Relative action... | 114 | $2 \times 100$ | $10 \times 72$ |

or a reduction of 40 per cent. per unit area exposed when the actual area was increased ten fold. But rough calculation shows that the same amount of copper used in these experiments, when sprayed on to a tree would be spread over a surface 6000 times greater in area than that of the one iron rod, and, even if the rate of action were thereby reduced to only one-hundredth of that observed in the case of the one rod, the actual amount of action occurring in unit time would be sixty times greater than with the rod. Thus we may get a very energetic absorption or fungicidal action, with a substance showing a minimum of solubility, though the possibility of this action depends entirely on the substance passing into solution. Of course, if the solid basic sulphate is separated from the liquid, the latter by itself will give no appreciable reaction with iron, just as Barker and Gimingham found that it had but little action on fungi, simply because there is very little copper in solution, but the fact that the presence of the solid sulphate in the

liquid is necessary for the production of an appreciable action in either case, is certainly no proof that the solid is directly acted upon either by the iron or the fungus; the solid merely serves to keep up the supply of dissolved copper in the liquid.

The experiment which Barker and Gimingham rely upon as establishing definitely their view that an insoluble copper compound is dissolved by excretion from the fungus, appears to be very unconvincing. A drop of basic sulphate liquid on a cover-slip was dried; a drop of liquid containing fungus spores was similarly dried on another slip, and partially superimposed on the patch of basic sulphate. The slips were then kept in a moist atmosphere for 24 hours, and it was found that the spores had germinated only in the region where there was no basic sulphate. It is difficult to see wherein the convincingness of this result lies: for it is just what would occur if the fungicidal action were due to the presence of soluble copper in the fungicide. That the result affords no proof of the excretion of a solvent substance from the spores, may be demonstrated by substituting for these a substance which certainly excretes no copper-dissolving body, namely, potassium ferrocyanide: a drop of a solution of this and of Woburn Bordeaux mixture are dried on separate pieces of paper, they are then partially superimposed, and pressed together, being kept moist by placing above them a slightly damped piece of paper. After a time the portions of the blotches which were in contact become red, whilst those portions which were not in contact remain unaffected. No action occurs if the papers are kept quite dry.

It is only necessary to add that if fungicidal action depended on a solvent material exuded from the fungus, all copper compounds, or at any rate all the basic sulphates and carbonates, would be equally efficacious for a given weight of copper present, a proposition which is contrary to all experience. Indeed ordinary Bordeaux mixture with excess of lime should be much more effective than other compounds with the same copper content, for the lime itself seems to have a conspicuous fungicidal action (Freeman, this *Journal*, III. 400); and this fungicidal action, it may be remarked, accounts for the results obtained by Barker and Gimingham (*loc. cit.* p. 86), when they found that ordinary Bordeaux was effective in an atmosphere free from carbon dioxide, which they accept as proving that carbon dioxide does not bring the copper of such a mixture into solution.

# THE DIRECT ASSIMILATION OF INORGANIC AND ORGANIC FORMS OF NITROGEN BY .HIGHER PLANTS.

By H. B. HUTCHINSON and N. H. J. MILLER.

(*Lawes Agricultural Trust.*)

In the earliest experiments on the assimilation of combined nitrogen the substances employed were, in addition to nitrates and ammonium salts, such organic compounds as occur in animal excrementitious matters and refuse, or are produced in their decomposition—urea, hippuric acid, trimethylamine, creatine, etc. Later on, when it became evident that any nitrogenous compound applied to the soil will, sooner or later, under ordinary conditions, be converted into nitrates, and so become available for plants, whether originally so or not, the question still retained its interest although from a different point of view. If it can be shown that from certain types of nitrogen compounds plants can directly obtain all the nitrogen they require, and that of such types some are more favourable than others, the results cannot fail to throw some light on the synthetical processes in plants. It must however be borne in mind that the substances supplied may undergo changes within the roots, or at the surface of the roots, and not reach the assimilating organs in their original forms.

In this connexion we would call attention to an important paper recently published by T. Takeuchi[1] who showed the presence of urease in several seeds. In experiments with urea and several other compounds it was found that the powdered seeds readily convert urea into ammonia and that biuret is slightly attacked, whilst the following compounds gave negative results: guanidine, arginine, benzamide, allantoin, leucine, alanine, tyrosine, creatine, histidine, guanine, glycine, uric and hippuric acids. The enzyme was found to be present in *Glycine hispida*, *Phaseolus vulgaris*, oats, paddy rice and buckwheat, but not in barley, upland rice, rye, maize, rape and radish.

[1] On the Occurrence of Urease in Higher Plants (*J. Coll. Agric. Tokio*, 1909, **1**. 1–14).

Further experiments in this direction seem very desirable as it is conceivable that the ability of a plant to utilise an organic nitrogen compound may depend on the presence of an enzyme capable of converting it into ammonia or perhaps some other less complex form.

With regard to the utilisation of nitrates by plants the various theories which have from time to time been suggested all assume the initial process to be a reduction. Gautier, in 1872, suggested that the nitric acid is first reduced by formaldehyde, and that hydrocyanic acid is the final product and the first form in which nitrogen is actually assimilated by combining with some carbohydrate. Treub carried the suggestion a step further and supposes that the hydrocyanic acid derived from nitrates reacts with formaldehyde and ammonia with production of amino-nitriles which yield amino-acids by hydrolysis

$$HCN + H \cdot CHO + NH_3 = NH_2 \cdot CH_2 \cdot CN + H_2O.$$
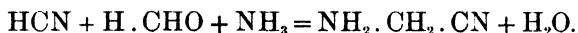
According to Bach, nitric acid is first reduced by formaldehyde to hydroxylamine, which with a further amount of formaldehyde gives formaldoxime. The latter, by intramolecular migration, is converted into formamide.

$$\overset{\displaystyle H}{\underset{}{\mid}}\atop{CHO} + NH_2 \cdot OH \; = \; \overset{\displaystyle H}{\underset{\displaystyle H}{\overset{\mid}{\underset{\mid}{C}}}} : N \cdot OH + H_2O$$

and

$$\overset{\displaystyle H}{\underset{\displaystyle H}{\overset{\mid}{\underset{\mid}{C}}}} : N \cdot OH \; = \; \overset{\displaystyle H}{\underset{}{\overset{\mid}{CO}}} \cdot NH_2$$
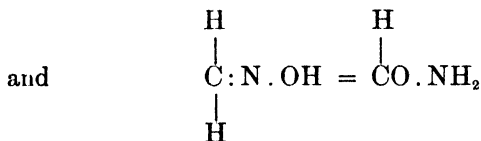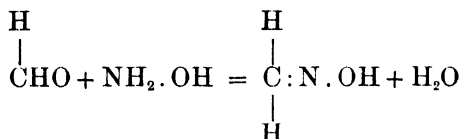
Baudisch[1] has recently shown that dilute solutions of potassium nitrate are converted into nitrite by exposure to diffused daylight, and that nitrites are reduced by methyl alcohol, in diffused light, to formhydroxamate

$$KNO_2 + CH_3 \cdot OH = OH \cdot CH : N \cdot OK + H_2O.$$

Formaldehyde and potassium hyponitrite are also formed. Baudisch also found that both nitrates and nitrites are readily reduced by aldehydes in presence of light with production first of hydroxamic acids, and then ammonia and amines. The reduction is also brought about

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[1] *Ber. deut. chem. Ges.* 1911, **44.** 1009.

under the influence of light by laevulose (with evolution of carbon monoxide) and other carbohydrates, and by phenols and naphthols.

Without going further into this question[1] it may be remarked that the above theories collectively involve the assumption that the following compounds are available to plants as sources of nitrogen: hydrocyanic acid, amino-nitriles, hydroxylamine, formaldoxime and formamide.

The more difficult question regarding the initial process in the fixation of elementary nitrogen in the root-nodules of leguminous and other plants and by independent organisms has received very little attention up to the present time; and the only evidence we have of nitrogen being brought into combination by purely chemical means at the ordinary temperature is that furnished by Loew's experiment[2] with platinum black and alkali in which it was shown that ammonium nitrite is produced.

According to Winogradsky the most probable explanation is that ammonia is formed by the action of nascent hydrogen. Whilst Gautier and Drouin suggest that the nitrogen is oxidised either to nitric or nitrous acid, Heinze[3] thinks it probable that nitrogen is at once brought into combination with a hydrocarbon and suggests that a salt of carbamic acid may be first formed, or that carbamic acid may be produced from cyanamide.

With regard to nitrogenous constituents of root-nodules[4] it may be mentioned that Stoklasa detected asparagine and that Sana[5] found *l*-asparagine and glycine in bean-nodules.

Before describing the experiments on the assimilation of the nitrogen of the various substances employed it will be desirable to show in some detail what has already been done, attention being confined chiefly to the more recent results obtained under sterilised conditions.

### Inorganic Compounds[6].

Experiments by V. Meyer and E. Schulze (27) and more recently by Lutz (49) showed that hydroxylamine is not assimilated by maize and barley. Suzuki (55) found that barley failed when supplied with

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[1] Compare H. Franzen, *Sitzungsber. Heidelberger Akad. Wiss.* 1910.

[2] *Ber. deut. chem. Ges.* 1890, **23.** 1447.

[3] *Landw. Jahrb.* 1906, **35.** 907.

[4] This *Journ.* 1909, **3.** 179.

[5] *Abstr. J. Chem. Soc.* 1910, ii. 993.

[6] For results obtained with ammonium salts see this *Journ.* 1909, **3.** 179; and E. Pantanelli and G. Severini: "Alcune esperienze sulla nutrizione azotata delle piante verdi con diversi sali d' ammonio" (*Staz. sper. agrar. Ital.* 1910, **48.** 449—544).

nitrogen in the form of sodium hydroxylamine disulphonate. Loew's (32) experiments with hydrazine sulphate and azoimide also gave negative results. Maeno (42) showed that amidosulphonic acid is injurious to barley and other plants.

In experiments with water plants W. Knop (5) obtained indications that nitrous oxide is assimilated. Still better results were obtained by employing a mixture of nitrous oxide and ethylene.

Birner and Lucanus (13), in 1866, showed that 0·25 per cent. potassium nitrite solutions are inimical to the life of oat plants, whilst Molisch (31) found that even 0·01 per cent. solutions may be injurious. When, however, bean plants were supplied with a 0·002 per cent. solution of potassium nitrite, it was found that the whole of the nitrite was taken up, and assimilated in from 5 to 20 hours, both the solutions and the plants failing to show any nitrite reaction. M. Schultz (57) showed that whilst solutions containing 0·5 per cent. of potassium nitrite are poisonous to plants more dilute solutions are equal to nitrates as a source of nitrogen; and that when light is deficient nitrites are more readily assimilated than nitrates. The injurious action of the stronger solutions of nitrites was found to vary with different plants, *Leguminosae* being less able to withstand their action than *Gramineae*.

Perciabosco and Rosso (72) have shown that maize will grow in sterilised 0·1 per cent. solutions of potassium nitrite, and that the produce contained a very distinctly higher percentage of nitrogen than plants grown with nitrate.

Mazé (74) in water culture experiments with maize supplied with potassium nitrite found that growth was considerably retarded at first. The final growth was however quite normal and the amount of dry matter produced equal to that obtained with nitrate.

*Organic Compounds.*

A large number of experiments were made by Lutz (48) in 1898 and since on the assimilation of various organic compounds containing nitrogen. The method employed was to grow various plants in sterilised sand, over dishes containing mercuric chloride covered by large bell jars. It was found that the sand remained uninfected throughout the experiments. As regards amines Lutz obtained the following results.

Methylamine was assimilated by *Cucurbita max.* and propylamine by *Cucumis prophet.*; maize and *Ipomaea purpurea* showed very slight gains of nitrogen with propylamine. Amylamine showed a gain of

6·6 mg. nitrogen with *Cucurbita* and dimethylamine a gain of 3·8 mg. with the same plant. Slight gains were obtained with butylamine and trimethylamine, whilst tetramethyl- and tetraethylammonium chlorides gave negative results: more recent experiments by Molliard (71) in which radishes were grown in ignited pumice with methyl-, ethyl-, propyl-, dimethyl- and trimethylamine chlorides gave negative results.

As regards cyanides the only recent experiments seem to be those of Suzuki (54) who showed that plants are destroyed in solutions containing as little as 0·001 per cent. of potassium ferrocyanide, and of Rana Bahadur (59) who found that sodium nitroprusside is not assimilated. Acetamide, which seems to be the only acid amide which has been employed, is assimilated according to Bente (23). Choline, in experiments by H. T. Brown (63) with excised barley embryos, was found to be assimilated; and Schreiner *et al.* showed that small amounts of choline are beneficial to wheat seedlings. Schreiner (66) found that neurine acts as a stimulant in solutions containing only 6 parts per million and that solutions containing 25 parts per million are very injurious.

Piperidine. Lutz found that *Cucumis melo* failed to assimilate nitrogen in this form.

Glycine was employed in the early experiments of Hampe, Knop and Wolf, and Wagner (21). Schreiner found that in solutions containing 0·1 per cent. or less it is beneficial to wheat seedlings. Hansteen (47) showed that *Lemna*, in absence of light, produces proteins from glycine and glucose, but not with sucrose.

Lutz obtained negative results with *Cnicus benedictus* and *Cucumis melo* supplied with glycollamide as source of nitrogen.

Betaine also gave negative results in experiments by Lutz with maize and *Ipomaea purpurea*. H. T. Brown, on the other hand, found that excised barley embryos gained 50 per cent. of nitrogen (as in the experiment with choline) with betaine; and Schreiner showed that solutions containing 5 per million were beneficial to wheat.

Alanine. Hansteen (47) obtained negative results in experiments with *Lemna*, in which the plants, kept in darkness, were supplied with alanine and glucose (or sucrose). Schreiner, Reed and Skinner found that 0·05 per cent. of alanine is toxic to wheat seedlings, whilst smaller amounts proved to be beneficial. In a series of experiments made in 1896 Prianischnikoff and Lebedeff (44) found that leucine is not assimilated by barley, and similar indications were obtained by Lutz, in 1899, with *Ipomaea purpurea*. Subsequent experiments by Lutz (61) with *Cucumis vulgaris* showed, however, gains of 0·636 and 0·723 mg. of

nitrogen corresponding with about 38 per cent., and 6·5 and 3·9 mg. of dry matter. The seeds germinated normally and the growth was vigorous. Hansteen found that *Lemna* grown in absence of light failed to produce protein from leucine and glucose (or sucrose); and, more recently, Brown showed that whilst excised barley embryos gained a slight amount of nitrogen when supplied with leucine as source of nitrogen, there was no gain, but rather a loss of dry matter. Schreiner, on the other hand, found that solutions containing 0·05 per cent. of leucine, or less, were uniformly beneficial to wheat.

The first experiment with urea made under sterilised conditions seems to be that of Prianischnikoff and Lebedeff (44) who obtained negative results. In 1897, Suzuki, who made a number of experiments with yellow lupins, potatoes, *Halesia hispida*, wheat and barley, found that, with the exception of barley, all the plants produced more asparagine from urea than from ammonium salts; and that, unlike nitrates, urea gives rise to the production of asparagine in etiolated shoots. Thomson (50) also showed that the nitrogen of urea is directly assimilated by oats and that the plants contained 50 per cent. more protein nitrogen, although only slightly more total nitrogen, than plants supplied with nitrates. Sawa (53) found that solutions containing more than 0·05 per cent. of urea are injurious. Hansteen's experiments with *Lemna* showed that protein is readily produced from urea in presence of glucose or sucrose.

Kawakita (58) showed that 0·464 gram of biuret per litre killed young barley plants in a few days.

Thiosinamine. The only experiment with this substance seems to be one by Knop and Wolf who found that it is toxic.

Kawakita (58) found that solutions containing 0·5 gram of guanidine in 250 c.c. killed young barley plants in three days and that solutions four times as dilute destroyed the plants in two weeks. Schreiner showed that even 1 per million of guanidine carbonate is highly injurious. Creatine was employed in 1869 by Wagner (21) who found that it is readily assimilated, and recent experiments by Skinner (75) show that both creatine and creatinine are assimilated by wheat. Hansteen showed that *Lemna*, in absence of light, is unable to produce protein from creatine and glucose or sucrose.

Dicyanodiamide is, according to Loew (67), assimilated by *Elodea* from 0·2 per cent. solutions. Perotti (68) obtained similar indications with cereals in water culture containing 0·5 gram of dicyanodiamide per litre, and in sand cultures; Aso (70) who grew buckwheat and oats

in solutions containing 0·01 per cent. of dicyanodiamide also found that it is assimilated.

Dicyanogen.   Loew (67) showed that barley plants are killed by solutions containing 0·01 per cent. of dicyanogen.

Aspartic acid.   Prianischnikoff and Lebedeff (44) obtained negative results, whilst Brown (63) showed that it is assimilated by excised barley embryos.   According to Schreiner, Reed and Skinner, wheat plants are killed by 0·05 per cent. of aspartic acid whilst 0·01 per cent. solutions are not toxic.

Baessler (26) showed in 1886 that asparagine is readily assimilated by maize provided that the roots do not remain too long in the solutions.   Otherwise they are liable to injury due, it is supposed, to the action of decomposition products.   Prianischnikoff and Lebedeff failed to obtain assimilation with oats in sterilised solutions.   On the other hand, Nakamura (43), using 1 per cent. solutions of asparagine, found that barley produced considerably more growth than with ammonium succinate.   The more recent experiments of Brown showed that excised barley embryos produced as much dry matter with asparagine as with nitrate whilst the percentage of nitrogen in the dry matter was much higher.   According to Hansteen (47), *Lemna* grown in darkness is able to produce protein from asparagine in presence of glucose but not with sucrose.   In experiments with wheat seedlings Schreiner found that 0·1 per cent. solutions of asparagine were beneficial.

Glutamic acid.   The only experiment with glutamic acid seems to be one by H. T. Brown who included it in the series already referred to.   The results with barley showed that the effect of glutamic acid was practically the same as with aspartic acid, asparagine, and nitrate, except that the percentage of nitrogen in the dry matter was rather lower than with nitrate.

Glutamine.   Hansteen found that glutamine, in presence of glucose, is converted into proteins by beans growing in absence of light, but not when sucrose is employed.   The result accords with that obtained with asparagine, whilst with glycine the presence of sucrose, and not glucose, seems to be essential.

Allantoin was found by Brown to give almost identical results with choline as source of nitrogen for excised barley embryos.

Alloxan.   Schreiner grew wheat seedlings in solutions of alloxan and found that whilst solutions containing 1—25 mg. per litre slightly stimulated growth, concentrations of 100 mg. per litre were toxic.

Uric acid was employed by S. W. Johnson (15) and by Hampe.

The more recent experiments by Thomson showed that it is assimilated by oats, but that its nutritive value is far less than that of urea.

Guanine.  Saturated solutions of guanine only contain 40 mg. per litre, and Schreiner found that solutions of that strength slightly promote the growth of wheat.

Xanthine is also very sparingly soluble.  Solutions containing 1—25 mg. per litre were found by Schreiner to be beneficial to wheat seedlings.

Caffein.  Lutz showed that maize, *Helianthus annuus* and *Cucurbita maxim.* do not assimilate caffein hydrochloride and that maize, in solutions of the free base, failed altogether.  Sawa, employing 0·1 and 0·25 per cent. solutions of caffein, also obtained negative results with celery.

Several experiments were made by Lutz with aniline, diphenylamine and benzylamine, but only negative results were obtained; and picric acid in an early experiment by Knop and Wolf also failed to give any result.

Hippuric acid.  Numerous experiments, commencing with those of S. W. Johnson (15) and Hampe (18), have been made with hippuric acid.  Of the more recent results those of Prianischnikoff and Lebedeff (44) failed to show assimilation, whilst Thomson obtained some growth with oats, but far less than with urea and nitrate.

Nitrobenzoic acid was employed by Knop and Wolf (11) in 1865 who obtained negative results.

Aminobenzoic acid.  Knop and Wolf failed to obtain assimilation.

Phenylalanine.  H. T. Brown (63) found that excised barley embryos supplied with phenylalanine as source of nitrogen only gained 10 per cent. dry matter.

Tyrosine.  Lutz (61) grew *Cucumis melo* in solutions containing tyrosine and failed to obtain any increase of nitrogen.  In later experiments, with *Cucumis vulgaris* he obtained normal germination and vigorous growth; in one experiment there was a gain of 0·211 mg. of nitrogen, or 11·9 per cent., and in a second experiment a gain of 19·5 per cent.  H. T. Brown found that excised barley embryos failed to assimilate nitrogen in the form of tyrosine, whilst Schreiner (66) showed that solutions containing 0·01 per cent., are very injurious to wheat seedlings and that even 0·001 per cent. solutions are toxic.

The remaining experiments with organic compounds have all given negative results.  Lutz attempted to grow maize and *Cucurbita max.* with naphthylamine hydrochloride and with pyridine, whilst Sawa found

that antipyrine is toxic to celery in 0·1 per cent. solutions. Scatole, pyridine, picoline, piperidine and quinoline were all found by Schreiner to be very toxic. The only experiments with alkaloids under sterilised conditions are those of Lutz who failed to obtain growth with atropine, cocaine, quinine and morphine.

### Humus.

In 1842 de Saussure (3) published some results of experiments on the assimilation of potassium humate by beans and *Polygonum persicaria*. The plants grew satisfactorily producing healthy roots, and reduced the amount of humus by from 6 to 43 mg. in a week or two; the solutions became considerably lighter in colour. From these results and the fact that the roots remained colourless, de Saussure drew the conclusion that humus is not only absorbed but assimilated. And he attributed the failure of similar experiments by Harting to the roots not having enough space to develop.

Wiegmann and Polstorff (4) soon afterwards (1845) grew *Mentha undulata* and *Polygonum persicaria* for a month in a humus solution. The plants grew well and one solution lost 16 mg. of humus. Some of the same solution exposed to air during the same period, without vegetation, lost 12 mg.

In Grandeau's (22) experiments with wheat and barley supplied with a neutral solution of ammonium humate, the solution became colourless owing to the precipitation and not to the assimilation of the humus. Grandeau supposes that the humus is dialysed by the roots, the organic matter remaining outside and the mineral matter being absorbed. As regards nitrogen it is probable that the ammonia of the ammonium humate was sufficient for the needs of the plants; and the ammonia being removed the free humic acid would of course be precipitated.

Bréal (37), in 1894, made experiments with *Poa annua*. A tuft of the grass was deprived of its roots and placed in water until new roots were formed. The tuft was then divided into two parts, one of which was supplied with a solution of potassium humate, whilst the roots of the other part were cut off and placed in a second portion of the same solution. In two or three days the humate solution was completely decolourised by the grass, whilst the solution containing the roots which had been cut off remained unchanged. In another experiment, similar to the ones made by Sachs to demonstrate the action of roots on marble,

etc., it was found that the roots of *Poa* acted in like manner on a paper filter covered with insoluble humic acid.

Wiley showed that oats grown in peat soil contained about 25 per cent. more nitrogen than when grown in ordinary arable soil, and that the excess of nitrogen was mainly in the form of amides and not proteins. The results indicate that a portion of the nitrogenous matter of peat is directly assimilated and that the nitrogen thus taken up is in the form of amides.

### SUMMARY OF RESULTS.

The results of all the experiments above described may be shortly summarised as follows.

Nitrites have been shown by Molisch, M. Schultz, and by Perciabosco and Rosso to be assimilated by various plants from sufficiently dilute solutions. Perciabosco and Rosso found that plants supplied with nitrogen in this form contain higher percentages of nitrogen than with nitrates as source of nitrogen. This is also the case, as was already shown, when plants take up all, or most, of their nitrogen as ammonium salts[1].

TABLE I. *Assimilation of the nitrogen of organic compounds.*

| | | | Dry matter | | Nitrogen | | |
|---|---|---|---|---|---|---|---|
| | | | Actual (gram.) | Gain (gram.) | In dry matter per cent. | Total (mgm.) | Gain (mgm.) |
| Methylamine ... | Lutz (48) | Cucurbita max. | 0·223 | 0·086 | 7·26 | 16·2 | 5·9 |
| Propylamine ... | ,, | Cucumis prophet. | 0·169 | 0·016 | 7·42 | 12·5 | 1·6 |
| Amylamine ... | ,, | Cucurbita max. | 0·195 | 0·058 | 8·65 | 16·9 | 6·6 |
| Dimethylamine ... | ,, | ,, | 0·210 | 0·073 | 6·70 | 14·1 | 3·8 |
| Diethylamine ... | ,, | ,, | 0·202 | 0·065 | 7·57 | 15·3 | 5·0 |
| Trimethylamine... | ,, | Maize | 0·162 | 0·028 | 3·40 | 5·5 | 1·0 |
| Choline ... ... | Brown (63) | Barley | 0·152 | 0·017 | 2·88 | 4·4 | 1·4 |
| Betaine ... ... | ,, | ,, | 0·135 | — | 3·34 | 4·5 | 1·5 |
| Urea ... ... | Thomson (50) | Oats | 2·227 | — | 4·63 | 103·0 | — |
| ,, ... ... | ,, | Barley | 1·810 | — | 5·21 | 94·0 | — |
| ,, ... ... | Frank | Lupins | 10·071 | — | 1·11 | 111·4 | 102·3 |
| Aspartic acid ... | Brown | Barley | 0·153 | 0·012 | 3·61 | 5·53 | 2·5 |
| Asparagine ... | Prianischnikoff (44) | ,, | 0·710 | — | — | — | — |
| ,, ... | Brown | ,, | 0·155 | 0·020 | 5·22 | 8·1 | 5·1 |
| Glutamic acid ... | ,, | ,, | 0·156 | 0·021 | 3·75 | 5·9 | 2·9 |
| Allantoin ... | ,, | ,, | 0·143 | 0·008 | 3·13 | 4·5 | 1·5 |
| Uric acid... ... | Thomson (50) | Oats | 1·177 | — | 3·67 | 4·1 | — |
| ,, ... ... | ,, | Barley | 1·691 | — | 3·37 | 5·7 | — |
| Hippuric acid ... | ,, | Oats | 0·472 | — | 2·90 | — | — |
| ,, ,, ... | ,, | Barley | 0·317 | — | 2·86 | — | — |

[1] This *Journ.* 1909, **3.** 191.

Experiments with other inorganic forms of nitrogen—amidosulphonic acid, hydroxylamine, hydrazine and azoimide—have all given negative results up to the present time.

As regards organic compounds the majority have given negative if not uncertain results. The results in which at least 1 milligram of nitrogen was assimilated are set out in Table I, p. 291.

### EXPERIMENTAL.

The methods employed in the following experiments were the same as described in the previous paper on the assimilation of ammonium salts[1]. The seeds were sterilised by means of a mercuric chloride solution under reduced pressure, and the plants (peas) were grown in Woulffe's bottles containing the following mineral substances in about 1 litre of water.

| | | |
|---|---|---|
| $CaSO_4$ ... ... | 0·5 gram. |
| $MgSO_4, 7H_2O$ ... | 0·5 ,, |
| KCl ... ... | 0·25 ,, |
| NaCl ... ... | 0·25 ,, |
| $KH_2PO_4$ ... ... | 0·5 ,, |
| $Fe_2Cl_6$ ... ... | Trace |

The nitrogenous compound was added in such quantity as to supply about 80 mg. of nitrogen.

Series I. *Peas in Water Cultures with various forms of Nitrogen.* The following compounds were employed:

1. Ammonium sulphate.
2. Ethyl nitrate.
3. Tetranitromethane.
4. Acetamide.
5. Propionitrile.
6. Alanine.
7. Urea.
8. Guanidine hydrochloride.
9. Sodium aspartate.
10. Barbituric acid.
11. Hippuric acid.
12. Peptone.

The seedlings were placed in the solutions in October 1908. A satisfactory beginning in either one or both bottles was obtained with ammonium sulphate, acetamide, alanine, urea, guanidine, sodium aspartate, hippuric acid and peptone, whilst ethyl nitrate proved

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[1] This *Journal*, 1909, **3**. 179,

unsatisfactory, and the plants supplied with tetranitromethane and with propionitrile failed altogether.

Owing, however, to the late time of the year the plants made comparatively little growth and by January all of them died off. In April 1909 the whole experiment was repeated with fresh solutions, when necessary, the only difference being that calcium carbonate was added to the solutions containing tetranitromethane.

Of the different substances employed urea gave the best results. With ammonium sulphate the amount of growth was a good deal less than in the previous series in which calcium carbonate was added. Acetamide gave more growth than ammonium sulphate, whilst with guanidine, peptone and sodium aspartate the growth was somewhat less, and with alanine distinctly less. With barbituric acid, propionitrile, ethyl nitrate and tetranitromethane the plants failed although they seemed to have taken up, if not assimilated, small amounts of nitrogen in most cases.

At the conclusion of the experiments all the solutions were found to be free from nitrates, and the whole of the nitrogen remaining in the ammonium sulphate bottles was found to be unchanged. The guanidine bottles gave indications of infection and this experiment was repeated in the next series.

Samples of the culture solutions were carried over to Omelianski's medium for the detection of nitrifying organisms and to nutrient- and urea-gelatin to test for the presence of organisms related to putrefactive and urea bacteria. All the solutions were shown to be free from nitrifying bacteria, while the occurrence of other organisms is indicated in Table II.

On the whole there were no great differences in the amounts of growth in the plants which remained alive except in the case of one of the urea plants which was distinctly better than any of the others, not only as regards leaf and stem, the roots being in every respect more normal (see photos, Plate V). The roots in the ammonium sulphate solution had about the same length as those of the urea plant but were less bushy.

Those of the sodium aspartate plant were very short, but very thick; whilst the acetamide plant had roots of great length, about 18 inches. The peptone plant was altogether small and produced very little root. As peptone has been shown to occur in various seedlings[1],

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[1] Mack, W. R., "Über das Vorkommen von Pepton in Pflanzensamen," *Inaug.-Diss.*, Leipzig, 1903.

it might be expected to be available as a source of nitrogen and the results in Table II indicate that a small amount has in fact been utilised by the pea plant. It seems possible, however, that whilst peptone and certain other nitrogen compounds may be taken up and to some extent utilised by plants, they may be unable to furnish the whole of the nitrogen required, or, at any rate, to supply the plant with sufficient rapidity.

TABLE II. *Peas growing in Water Cultures,* 1909.

| | | Nitrogen in Solutions | | Dry produce | Nitrogen | | |
|---|---|---|---|---|---|---|---|
| | | at commencement | at conclusion | | in dry subst. | total in dry subst. | gain or loss [1] |
| | | mgm. | mgm. | gm. | p.c. | mgm. | mgm. |
| Ammonium sulphate | a | 80 | 68 | 0·368 | 5·250 | 19·3 | 8·9 |
| | b | 80 | 65·5 | 0·451 | 5·075 | 22·9 | 12·5 |
| Ethyl nitrate | a | 80 | — | — | — | 14·6 | 4·2 |
| | b | 80 | — | — | — | 12·3 | 1·9 |
| Tetranitromethane + CaCO₃ ... | a | 80 | — | — | — | 14·8 | 4·4 |
| | b | 80 | — | — | — | 13·5 | 3·1 |
| Acetamide | a | 78 | 61 | 0·696 | 3·701 | 25·8 | 15·4 |
| | b | 78 | — | — | — | 12·3 | 1·9 |
| Propionitrile | a | 80 | — | — | — | 14·5 | 4·1 |
| | b | 80 | — | — | — | 11·3 | 0·9 |
| α-Aminopropionic acid | a | 79 | 74 | 0·269 | 5·777 | 15·5 | 5·1 |
| | b | 79 | — | — | — | 7·8 | - 2·6 |
| Urea | a | 80 | 58 | 0·533 | 5·358 | 28·6 | 18·2 |
| | b² | 80 | 43 | 0·758 | 5·818 | 44·1 [2] | 33·7 |
| Guanidine hydrochloride | a | 75·5 | 63 | 0·301 | 5·930 | 17·9 | 7·5 |
| | b² | 75·5 | 64 | 0·425 | 4·447 | 18·9 [2] | 8·5 |
| Sodium aspartate | a² | 81 | 71 | 0·367 | 5·188 | 19·0 | 8·6 |
| | b² | 81 | 73·5 | 0·264 | 6·841 | 18·1 [2] | 7·7 |
| Barbituric acid | a | 80 | — | — | — | 16·3 | 5·9 |
| | b | 80 | — | — | — | 9·7 | - 0·7 |
| Hippuric Acid | a | 80 | — | — | — | 8·9 | - 1·5 |
| | b | 80 | 78 | 0·299 | 3·629 | 10·9 | 0·5 |
| Peptone | a | 98 | — | — | — | 8·7 | - 1·7 |
| | b | 98 | 94 | 0·329 | 4·553 | 15·0 | 4·6 |

Series II. *Peas in Water Cultures with Organic Nitrogen.* These experiments were in all respects similar to the last series.

The substances employed were:

[1] Deducting 10·4 mg. which was the average amount present in the seed. The variations in the individual seeds amounted to 4 mg.

[2] Culture infected.

1. Trimethylamine hydrochloride.
2. Methyl carbamate.
3. Guanidine hydrochloride.
4. Cyanuric acid and $CaCO_3$.
5. Oxamide.
6. Humus.

The humus solution was prepared from garden soil and was neutralised as nearly as was possible without precipitating. The seedlings were placed in the solutions by July 10, 1909. The plants all made a good start with the exceptions of those supplied with methyl carbamate, one of which failed altogether. The humus plants, which finally gave the best results, were distinctly behind the other plants during the first two or three weeks.

The guanidine plants were the first to lose their colour, but by August 17 most of the plants had dried up except those in humus solution and those supplied with cyanuric acid and trimethylamine, and the latter had very little green remaining.

On taking out the plants the following root measurements were made (in cm.).

|   | $a$ | $b$ | av. |
|---|---|---|---|
| 1. | 25 | 27 | 26 |
| 2. | 6 | 11 | 8·5 |
| 3. | 19 | 22 | 20·5 |
| 4. | 33 | 29 | 31 |
| 5. | 37 | 24 | 30·5 |
| 6. | 29 | 25 | 27 |

The bacteriological examination of the solutions showed that the conditions of sterilisation had been maintained to the end.

The results set out in Table III show that the peas failed to utilise trimethylamine and methyl carbamate. Both these compounds are of interest as possible sources of nitrogen. Trimethylamine is known to occur in a small number of plants, and as a constituent of such substances as choline, betaine, caffeine and the lecithins etc. is very widely distributed; whilst as a constituent, or decomposition product, of fish manure it must frequently come into contact with the roots of plants. As regards carbamic acid, it has been suggested by Heinze[1] that this may be one of the first compounds produced when free nitrogen is brought into combination by nitrogen-fixing organisms. It is possible, of course, that the methyl salt employed is an unfavourable form in which to supply the compound for consumption by plants.

[1] *Landw. Jahrb.* 1906, **35**. 907.

There is, however, no choice since the metallic salts are too unstable to undergo the process of sterilisation.

TABLE III.    *Peas grown in Water Cultures, 1909.*

| | Nitrogen in Solutions | | Dry produce | Nitrogen | | |
|---|---|---|---|---|---|---|
| | at commencement | at conclusion | | in dry subst. | total in dry subst. | gain or loss[1] |
| | mgm. | mgm. | gm. | p.c. | mgm. | mgm. |
| Trimethylamine hydrochloride... *a* | 81·4 | 73·7 | 0·273 | 3·97 | 10·9 | 0·5 |
| *b* | 81·4 | 74·0 | 0·190 | 3·42 | 6·5 | – 3·9 |
| Methyl carbamate ................ *a* | 60 | 65·7 | 0·109 | 4·04 | 4·4 | – 6·0 |
| *b* | 60 | 65·2 | 0·205 | 3·28 | 6·7 | – 3·7 |
| Guanidine hydrochloride ......... *a* | 75·3 | 74·1 | 0·232 | 4·50 | 10·4 | 0 |
| *b* | 75·3 | 70·0 | 0·321 | 4·08 | 13·1 | 2·7 |
| Cyanuric acid + CaCO₃ ........... *a* | 84·9 | 82·4 | 0·318 | 3·68 | 11·7 | 1·3 |
| *b* | 84·9 | 85·0 | 0·161 | 3·17 | 5·1 | – 5·3 |
| Oxamide ......................... *a* | 52·4 | 46·4 | 0·312 | 4·49 | 14·0 | 3·6 |
| *b* | 52·4 | 51·2 | 0·242 | 3·62 | 8·8 | – 1·6 |
| Humus ........................... *a* | — | 54·9 | 0·577 | 2·83 | 16·3 | 5·9 |
| *b* | — | 54·6 | 0·449 | 3·32 | 15·0 | 4·6 |

Guanidine hydrochloride was assimilated to a slight extent by one plant. Previous experiments had always given negative results with guanidine probably owing to the strongly alkaline base having been employed, or to too great concentration of the solutions. Oxamide was also assimilated in one case; whilst humus gave much the best results of any in this series.

Series III. *Peas in Water Cultures with different forms of Nitrogen, chiefly organic.* The culture solutions contained in addition to the usual mineral nutrients, as previously described, the following compounds as sources of nitrogen:

1 *a* and *b*.  No Nitrogen.
2 *a* and *b*.  Formamide.
3 *a* and *b*.  Glycine.
4 *a* and *b*.  Hexamethylenetetramine.
5 *a* and *b*.  Hydroxylamine hydrochloride.
6 *a* and *b*.  *para*-Urazine.
7 *a* and *b*.  Alloxan.
8 *a* and *b*.  Barbituric acid and Calcium carbonate.

There were also two bottles, 9*a* and 9*b*, containing garden soil heated at 120° for two hours.

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¹ See footnote on p. 294.

Sterilised pea seedlings were placed in the first six pairs of bottles in July 1910, and in the last three pairs in the following September.

TABLE IV.  *Peas grown in Water Cultures*, 1910.

| | | | Dry matter | | Nitrogen | | |
|---|---|---|---|---|---|---|---|
| | | | Total | Gain ±0·025 | In dry substance | Total | Gain or loss[1] |
| | | | gm. | gm. | p.c. | mgm. | mgm. |
| 1 | Without Nitrogen | {a | 0·210 | − 0·065 | 4·72 | 9·9 | − 0·5 |
| | | {b | 0·200 | − 0·075 | 5·38 | 10·35 | — |
| 2 | Formamide | {a | 0·320 | 0·045 | 4·42 | 14·1 | 3·7 |
| | | {b | 0·410 | 0·135 | 4·60 | 18·9 | 8·5 |
| 3 | Glycine | {a | 0·350 | 0·075 | 4·62 | 16·2 | 5·8 |
| | | {b | 0·150 | − 0·125 | 5·96 | 8·9 | − 1·5 |
| 4 | Hexamethylenetetramine | {a | 0·170 | − 0·105 | 5·38 | 9·2 | − 1·2 |
| | | {b | 0·180 | − 0·095 | 7·30 | 13·1 | 2·7 |
| 5 | Hydroxylamine hydrochloride | {a | 0·190 | − 0·085 | 4·88 | 9·3 | − 1·1 |
| | | {b | 0·210 | − 0·065 | 5·59 | 11·7 | 1·3 |
| 6 | *para*-Urazine | {a | 0·252 | − 0·023 | 5·61 | 14·1 | 3·7 |
| | | {b | 0·166 | − 0·109 | 5·46 | 9·1 | − 1·3 |
| 7 | Alloxan | {a[2] | 0·318 | 0·043 | 4·60 | 14·6 | 4·2 |
| | | {b | 0·467 | 0·192 | 3·73 | 17·4 | 7·0 |
| 8 | Barbituric acid and calc. carb. | {a[2] | 0·278 | 0·003 | 5·69 | 15·8 | 8·4 |
| | | {b | 0·698 | 0·423 | 3·92 | 27·4 | 17·0 |
| 9 | Garden soil | {a[2] | 0·367 | 0·092 | 4·48 | 16·5 | 6·1 |
| | | {b[2] | 0·475 | 0·200 | 4·68 | 22·2 | 11·8 |

The plants made a good start on the whole, but with the exception of those supplied with alloxan and barbituric acid and the ones growing in garden soil, there was not very much final growth. The results (see Table IV) show however that formamide and glycine were undoubtedly assimilated, although less readily than alloxan and barbituric acid, whilst the results obtained with hydroxylamine, hexamethylenetetramine and *para*-urazine are doubtful, as although one plant in each case contained more nitrogen than the original seed (which might be due to absorption without assimilation), there was a decided loss of dry matter.

The garden soil experiment was inconclusive as it was found that the soil in both bottles had become infected and contained considerable amounts of ammonia. It is perhaps worthy of note that, in the case of plants supplied with barbituric acid and alloxan, a slightly greater assimilation occurred where the sterile conditions were maintained throughout the experiment, than where infection occurred.

[1] See footnote on p. 294.                    [2] Infected.

Summary and Conclusions.

The various compounds may be divided into the following groups according to their availability, or otherwise, as direct sources of nitrogen for peas:

I.  Readily assimilated:
    Ammonium salts.
    Formamide.
    Acetamide.
    Urea.

    Barbituric acid $CO \begin{array}{c} \diagup NH.CO \diagdown \\ \diagdown NH.CO \diagup \end{array} CH_2.$

    Alloxan $CO \begin{array}{c} \diagup NH.CO \diagdown \\ \diagdown NH.CO \diagup \end{array} CO.$

    Humus.

II. Assimilated:
    Glycine.
    α-Aminopropionic acid.
    Guanidine hydrochloride.

    Cyanuric acid $CO \begin{array}{c} \diagup NH.CO \diagdown \\ \diagdown NH.CO \diagup \end{array} NH.$

    Oxamide.
    Peptone.

III. Doubtful or not assimilated:
    Hippuric acid.
    Trimethylamine.

    *para*-Urazine $CO \begin{array}{c} \diagup NH.NH \diagdown \\ \diagdown NH.NH \diagup \end{array} CO.$

    Hexamethylenetetramine.
    Ethyl nitrate.
    Propionitrile.
    Hydroxylamine hydrochloride.
    Methyl carbamate.

IV. Toxic:
    Tetranitromethane.

The results so far obtained are not sufficiently numerous to make it possible to trace any connexion between the assimilability or the reverse of the nitrogenous compounds and their constitution. Apart from humus which, as a mixture, cannot be said to have a constitution, the best results were obtained with urea and barbituric acid the former assimilating rather more nitrogen than the latter whilst barbituric acid gave the greater amount of dry produce. The similarity of the two results is probably to be accounted for by the fact that barbituric acid is readily decomposed with production of urea and malonic acid.

The next best result was obtained with acetamide which gave

nearly as much dry produce as barbituric acid, although less nitrogen was assimilated. After acetamide the highest results as regards the amounts of nitrogen assimilated were those obtained with ammonium sulphate (without calcium carbonate), formamide and alloxan which assimilated 12·5, 8·5 and 7 mg. of nitrogen respectively. Glycine, alanine and peptone come next with 5·8, 5·1 and 4·8 mg. of nitrogen. Then oxamide (3·6) and guanidine (2·7 mg. nitrogen) and lastly cyanuric acid.

With humates the nitrogen assimilated amounted to only 5·9 mg.; the amount of dry produce was however comparatively high being more than was obtained with urea.

The above grouping is of course only provisional and applies only to peas. It is quite possible that other plants may be able to utilise some of the substances which with peas have given negative results.

The ability of a plant to utilise the nitrogen of any particular compound will depend not only on the power of detaching the nitrogen but on the nature of the carbon compound or compounds remaining.

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EXPLANATION OF PLATE V.

Pea plants supplied with nitrogen in the form of

1. Ammonium sulphate.
2. Urea.
3. Acetamide.
4. Guanidine hydrochloride.
5. Sodium aspartate.
6. Peptone.

1

2

3

4

5

6

# THE FLOW OF WATER AND AIR THROUGH SOILS.

BY J. WALTER LEATHER, PH.D., F.I.C.

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IN Part I of the current volume of this Journal Green and Ampt make certain assumptions regarding the factors which control the rate of flow of water and air through soils, the basis of which is that the channels in a soil may be regarded as a bundle of capillary tubes, and that the velocity of flow of a liquid will consequently conform to Poiseuille's formula. Such an assumption cannot however be maintained. Poiseuille's formula was deduced from the ascertained rate of flow of liquids through straight tubes of approximately uniform diameter ; the latter being ·1 mm. or so. The formula is not however really accurate even under these limited conditions, as was shown by Thorpe and Roger[1], who found it necessary to add the correction $-\rho V/8\pi lt$. But the passages through the soil are not straight or of uniform diameter for any measurable distance, in fact they are wholly irregular, which Green and Ampt recognise, for they describe them as "irregular in area, length, direction and shape." But this being the case, Poiseuille's formula obviously cannot be expected to apply.

It is of course true that, having a certain cylinder full of soil, thoroughly saturated with water and through which water is flowing, the channels may assume fixed shape, and the velocity of flow would accordingly become uniform. Moreover, it is not difficult to fill a series of cylinders with portions of the same soil so nearly uniformly alike that the rate of flow of water through each will approximate to its neighbour. Therefore the agreement among the experimental results which Green and Ampt obtained with several portions of the same soil is not surprising. But this does not make their primary assumption legitimate. It is also to be noted that their experiments

[1] *Phil. Trans.* 1894, A, Part II.

showed that the flow of water through any of the three directions adopted, did not in fact accord with the expectation which was arrived at from Poiseuille's formula.

The most interesting case which was tested is the flow through the horizontal column of soil from a water-reservoir. Here the acting force is simply the tension of the advanced water surface, and here they found that the relation between the mass of water absorbed and the time, was a logarithmic one. Thus they arrive at a result similar to that which I deduced some years ago and dealt with in Memoir No. VI of the Chemical Series of the Imperial Department of Agriculture.

But as regards the downward movement of water through soils during wet weather, *i.e.* agricultural drainage, I venture to refer to the explanation of its nature which has been published in Memoir No. X of the same series. The process cannot be likened to water flowing downwards through tubes. The advanced portion of the water spreads out in much the same manner as indicated by the curve, Fig. 3, in Green and Ampt's communication; the water forms a "wave," the "length" of which constantly increases whilst the "height" diminishes, unless the rainfall is very heavy and continuous.

# THE INFLUENCE OF TIME OF CUTTING UPON THE YIELD AND COMPOSITION OF HAY.

By CHARLES CROWTHER, M.A., Ph.D.

AND

ARTHUR G. RUSTON, B.A., B.Sc.

(*Department of Agriculture, The University, Leeds.*)

THE variation in the chemical composition of fodder plants with advancing maturity has been the subject of numerous investigations on the Continent, but has received little attention in this country[1], despite its close bearing upon the annually recurring problem as to when the hay crop should be cut in order to obtain the best results.

There is no reason to doubt that the general conclusions drawn from the continental work are broadly applicable to the fodder crops grown in Great Britain but, in view of the great differences in climatic and soil conditions and the great influence which these factors exercise upon the growth of plants, there is obviously room for considerable variation in detail. It may be doubted, for instance, whether the changes in the composition of fodder plants growing under normal climatic conditions here will take place with such rapidity as has frequently been found to be the case on the Continent, where a delay of only a few days in cutting has apparently led to a serious depreciation in the value of the crop[2]. In other respects also there are such possibilities of variation that we have thought it desirable to place on record the results of comparative tests made by us with " seeds " hay grown in 1909 and 1910 at the Manor Farm, Garforth, Yorkshire. The hay was in each case the produce of a seeds mixture of the subjoined composition, sown with a corn crop in the preceding spring at the rate of 18 lb. per acre :

[1] Voelcker, *Journ. Roy. Agr. Soc.* 2nd Ser. III. 41 (1867).

[2] Wolff, *Landw. Jahrb.* (1879) VIII. 1, Suppl. pp. 34, 78.

| Perennial Rye Grass 2 parts; | Alsike | 3 parts |
|---|---|---|
| Italian Rye Grass 2 ,, | English Single-Cut Cowgrass | 1 part |
| White Clover 4 ,, | Chilian Red Clover | 1 ,, |
| Trefoil 4 ,, | Ribgrass | 1 ,, |

The two hay crops were grown in fields similarly situated near each other, and having soils of similar character, as may be seen from the analyses:

### Composition of Soils.

|  | Field 114 1909 Crop | Field 112 1910 Crop |
|---|---|---|
| *Mechanical Analysis* | | |
|  | per cent. | per cent. |
| Hygroscopic moisture | 1·35 | 1·32 |
| Fine gravel | 2·3 | 8·6 |
| Coarse sand | 41·0 | 42·1 |
| Fine sand | 38·9 | 22·8 |
| Silt | 8·2 | 10·4 |
| Fine silt | 5·1 | 8·5 |
| Clay | 2·0 | 2·3 |
| Matter dissolved by Acid, etc. | 0·6 | 1·1 |
| *Chemical Analysis* (Air-dried Fine Earth) | | |
| Loss on ignition | 4·91 | 4·76 |
| Silica, insoluble | 86·32 | 85·27 |
| ,,     soluble | 0·20 | 0·26 |
| $Fe_2O_3 + Al_2O_3$ | 5·76 | 6·43 |
| CaO, total | ·39 | ·53 |
| $CaCO_3$ | ·14 | ·21 |
| MgO | ·12 | ·47 |
| $P_2O_5$, total | ·29 | ·16 |
| ,,     available | ·049 | ·008 |
| $K_2O$, total | ·39 | ·28 |
| ,,     available | ·013 | ·012 |
| $SO_3$ | ·09 | ·08 |
| Nitrogen | ·127 | ·115 |

The soil of Field 112 was evidently appreciably richer in lime and magnesia, and poorer in available phosphates than the soil of Field 114, but otherwise there are no significant differences.

### 1909 Crop.

The seeds mixture from which this crop was derived was drilled on April 22, 1908, Tartar King oats having been sown ten days earlier. On May 11, 1909, a manurial dressing was given consisting of nitrate of soda (1 cwt.), superphosphate (2 cwts.), sulphate of potash (½ cwt.) and muriate of potash (¼ cwt. per acre). The whole of the field was cut on June 10, 1909, with the exception of a piece about 11 yards

square, very uniformly covered with crop. From one side of this a portion 10 yds. × 2 yds. was then cut carefully by hand, the produce collected at once, weighed, and dried under cover, mechanical losses being reduced to a minimum. When air-dry the weight of the hay was ascertained.

A second portion, 10 yds. × 2 yds., of the reserved piece was cut on June 28, and the produce dealt with similarly; whilst further similar plots, each 20 sq. yds., were cut on July 15 and Aug. 3 respectively. The weights of air-dry hay obtained at each cutting are given below:

| Date of cutting | Yield of hay[1] | |
|---|---|---|
| | Per plot | Per acre (to nearest cwt.) |
| | lbs. | cwts. |
| June 10 | 15 | 32 |
| „ 28 | 19½ | 42 |
| July 15 | 22¼ | 48 |
| Aug. 3 | 23⅓ | 50 |

At the first cutting the rye-grass was in full flower whilst there was a good bottom growth of the leguminous plants. Practical opinion was unanimous in condemning the cutting as too early, the second and third cuttings being regarded as more in accord with the normal practice of the district. At the second cutting the rye-grasses were distinctly taller, and seed-formation was actively in progress whilst the clovers were beginning to flower. At the third cutting the grasses were ripening and the clovers were in full bloom, whilst at the last cutting the whole crop was decidedly ripe.

The following table gives an indication of the general meteorological conditions during the active growing period and during the intervals between the respective cuttings:

| Period | Rainfall inches | Sunshine hours | Average temp. ° C. | Increase of crop lbs. dry matter per acre |
|---|---|---|---|---|
| April | 2·40 | 199 | 45° | |
| May | 2·13 | 226 | 49° | |
| June 1—14 | 0·12 | 81 | 50° | |
| „ 14—28 | 3·52 | 42 | 53° | 950 |
| June 28—July 15 | 1·68 | 63 | 56° | 620 |
| July 15—Aug. 3 | 2·63 | 75 | 58° | 300 |

[1] The results obtained with duplicate plots in the following year (p. 314) give an indication of the margin of error that should be allowed for in interpreting the results.

It will be observed that a fairly sunny and moist April and May were followed by a cool and—after the middle of June—moist June and July. The average temperature, though somewhat low, rose steadily throughout the whole period. The conditions were thus favourable for steady and prolonged growth, which is evidenced by the substantial increases of crop obtained between the different cuttings.

## Analysis of Hay.

For the purposes of analysis the whole crop of hay from each of the four plots was passed separately through a chaff-cutter, with the blades set to give the finest cut. Three separate samples of two or three pounds each were then drawn from the well-mixed chaff, or twelve samples in all. Before analysis each sample was reduced to a finely-divided condition in the laboratory. The portions actually used for the analyses were in every case drawn from the whole sample by a process of quartering.

The estimations commonly included in the analysis of feeding-stuffs were made, together with estimations of true protein (from nitrogen precipitated by cupric hydrate[1]), and pentosans (by distillation with hydrochloric acid and separation as furfural hydrazone), whilst a partial. analysis of the ash was also carried out. The results are summarised below. For convenience of comparison they are expressed as percentages of the dry matter in each case. The percentages of dry matter in the air-dried hays are also given so that the composition of the hay can be readily ascertained if desired.

The results tabulated for the first and second cuttings represent in each case the means of duplicate analyses of the three samples drawn from the chaff-cutter. In all subsequent cases the three samples were mixed together and a triplicate analysis made of the combined sample after comminution. The general lowness of the probable errors indicates that these methods were sufficiently reliable for the purpose.

The chief points of interest in the results are briefly:

(*a*) The steadily increasing proportion of crude fibre throughout the whole eight weeks.

(*b*) The gradual fall in the proportion of "amides" up to the third cutting, after which the reduction was very pronounced.

(*c*) The fall in the proportion of true protein during the moist

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[1] Barnstein, *Landw. Versuchsst.* 1900, **54**, 327.

latter half of June, followed by steady rise up to the period of the fourth cutting.

The true protein formed a steadily increasing proportion of the crude protein, the proportions in the hays obtained at the different cuttings being 70·1, 72·9, 72·8 and 85·3 per cent. respectively.

| | 1st Cutting (June 10) | 2nd Cutting (June 28) | 3rd Cutting (July 15) | 4th Cutting (Aug. 3) |
|---|---|---|---|---|
| | Percentage in air-dry hay | | | |
| Total dry matter................. | 88·65 + ·07 | 88·66 ± ·09 | 89·06 ± ·09 | 89·79 ± ·14 |
| | Percentage in dry matter | | | |
| True protein  ................... | 10·28 ± ·11 | 9·27 ± ·06 | 9·67 ± ·04 | 10·19 ± ·01 |
| " Amides " ................. | 4·36 ± ·13 | 3·45 + ·09 | 3·62 ± ·11 | 1·76 ± ·16 |
| Ether extract ................. | 2·65 ± ·12 | 1·64 ± ·04 | 1·58 ± ·03 | 1·47 ± ·02 |
| ¹Ash ................................ | 8·82 ± ·26 | 7·90 ± ·11 | 8·81 ± ·05 | 9·61 ± ·14 |
| Crude fibre ..................... | 27·3  + ·3 | 30·6  ± ·2 | 31·7  + ·2 | 34·4  ± ·2 |
| Sol. Carbohydrates and other matters not estimated ...... | 46·6 | 47·1 | 44·6 | 42·6 |
| Pentosans  ..................... | 20·7  + ·3 | 22·2  + ·3 | 20·5  ± ·4 | 24·0  ± ·3 |
| | Percentages in ash | | | |
| ¹Including SiO₂ ................. | 15·3 | 18·6 | 26·4 | 25·9 |
| K₂O ................. | 14·2 | 12·7 | 12·4 | 11·2 |
| CaO ................. | 9·4 | 8·6 | 9·6 | 8·0 |
| P₂O₅ ................. | 6·7 | 4·0 | 3·4 | 4·1 |

(d)  The gradual reduction in the proportion of " carbohydrates " after the second cutting.

(e)  The relatively high proportion of pentosans throughout the whole period.

(f)  The increasing richness of the ash in silica, whilst the proportions of $K_2O$ and $P_2O_5$ tended to fall.

In many ways a clearer indication of the history of the crop is given by the absolute weights of each ingredient produced upon a given area, as tabulated below for the crops per acre.

It will be noted that there was a steady increase throughout in the weights of total dry matter, true protein, crude fibre, pentosans, total ash, silica, lime and potash; that the weight of " amides " fell after the third cutting, and of " soluble carbohydrates " after the second cutting.  Attention may be specially directed to the rapid rate of increase in crude fibre and silica.

| | Pounds per acre | | | | Increase [or decrease (−)] over 1st Cutting | | |
|---|---|---|---|---|---|---|---|
| | 1st Cutting | 2nd Cutting | 3rd Cutting | 4th Cutting | 2nd Cutting | 3rd Cutting | 4th Cutting |
| | | | | | per cent. | per cent. | per cent. |
| Total dry matter......... | 3220 | 4180 | 4800 | 5100 | 30 | 49 | 59 |
| Crude protein ........... | 470 | 535 | 635 | 610 | 14 | 35 | 30 |
| True protein ........... | 330 | 390 | 465 | 520 | 18 | 41 | 57 |
| "Amides" .............. | 140 | 145 | 170 | 90 | 3 | 21 | −36 |
| Ether extract ........... | 85 | 70 | 75 | 75 | −18 | −12 | −12 |
| Crude fibre[1] .............. | 880 | 1280 | 1520 | 1760 | 45 | 73 | 100 |
| Pentosans[1] ............. | 670 | 930 | 1160 | 1230 | 39 | 73 | 84 |
| Sol. Carbohydrates excluding pentosans... | 830 | 1040 | 980 | 940 | 25 | 18 | 13 |
| Ash ...................... | 285 | 330 | 425 | 490 | 16 | 49 | 70 |
| SiO₂ ...................... | 43 | 61 | 112 | 127 | 42 | 160 | 195 |
| CaO ...................... | 27 | 28 | 40 | 39 | 4 | 48 | 44 |
| P₂O₅ ...................... | 18 | 13 | 15 | 20 | −28 | −17 | 11 |
| K₂O ...................... | 41 | 42 | 42 | 55 | 2 | 2 | 34 |

No attempt was made to estimate the amount of loss of dry matter that took place during the drying of the hay, but this was probably quite inappreciable.

### *Digestibility of Hay.*

In the absence of facilities for conducting digestion trials it was not possible to obtain more than an approximate estimate of the digestibility of the hay obtained at the different cuttings.

The digestible protein can, however, be estimated in the laboratory by means of an acid solution of pepsin, and such estimations were made on the lines of the Wedemeyer method[2].

No such laboratory method is available for the estimation of digestible carbohydrates and fibre, but in the case of coarse fodders their sum may be arrived at with fair accuracy by Henneberg and Stohmann's rule applying to digestion by ruminants[3], viz. that the total " soluble carbohydrates, etc." of a coarse fodder as estimated by chemical analysis are equal in amount to the sum of the digested " carbohydrates " and fibre. Kellner[4] quotes numerous data which lend strong support to this rule.

The ether extract is so trifling in amount that there is no appreciable

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[1] Some of the pentosan matter would be included in the crude fibre so that these two sets of figures overlap to some extent.

[2] *Landw. Versuchsstationen*, 1899, **51**, 383.

[3] Henneberg and Stohmann, *Neue Beiträge zur Begründung einer rat. Fütterung der Wiederkäuer*, 340 (1870).

[4] Kellner, *Die Ernährung der landw. Nutztiere.* 4te Aufl. S. 40.

error in assuming it to have been equally digestible in each sample, say 50 per cent.[1]

The "amides" may be regarded as completely digestible.

Proceeding upon these lines the following data were arrived at for the digestibility of the hays, and the weights of digestible matter per acre obtained at each cutting:

| | Per cent. in dry matter | | | | Weight per acre | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st Cutting $^{o}/_{o}$ | 2nd Cutting $^{o}/_{o}$ | 3rd Cutting $^{o}/_{o}$ | 4th Cutting $^{o}/_{o}$ | 1st Cutting lbs. | 2nd Cutting lbs. | 3rd Cutting lbs. | 4th Cutting lbs. |
| Crude protein ...... | 12·84 | 10·65 | 10·86 | 8·27 | 415 | 445 | 515 | 422 |
| True protein ...... | 8·48 | 7·20 | 7·24 | 6·51 | 275 | 300 | 345 | 332 |
| "Amides" ......... | 4·36 | 3·45 | 3·62 | 1·76 | 140 | 145 | 170 | 90 |
| Sol. Carbohydrates and fibre ......... | 46·6 | 47·1 | 44·6 | 42·6 | 1500 | 1970 | 2140 | 2180 |
| Ether extract ...... | 1·3 | 0·8 | 0·8 | 0·7 | 43 | 35 | 38 | 38 |

It will be noted that the crop became steadily less digestible as growth advanced, the maximum yield of valuable nutritive matters being attained at the third cutting.

From the proportions of digestible nutrients the relative nutritive values of the different hays either for maintenance or for productive purposes can be estimated. The "starch equivalent" for maintenance purposes is arrived at from the expression:

$$(\text{Digest. Protein} \times 1\cdot25) + (\text{Digest. "Oil"} \times 1\cdot9)$$
$$+ \text{Dig. Carbohyd}^s + \text{Dig. Fibre} + (\text{Amides} \times 0\cdot6),$$

the expression being based upon the relative physiological heat-values of the different nutrients[2].

The "starch-equivalents" for productive purposes are arrived at by Kellner's rules[3], summarised in the expression:

$$[(\text{Dig. Protein} \times 0\cdot9) + (\text{Dig. "Oil"} \times 1\cdot9)$$
$$+ \text{Dig. Carboh.} + \text{Dig. Fibre}] - [\text{Total Fibre} \times 0\cdot6].$$

The values arrived at in these ways are given below:

[1] Kellner's averages for meadow hay and red clover hay of medium quality are 51 $^{o}/_{o}$ and 53 $^{o}/_{o}$ respectively.

[2] Kellner, loc. cit. 84, 86, 88, 93.

[3] loc. cit. 580, 581.

*Starch-Equivalents of Hays.*

| | Per 100 lbs. dry matter | | Total crop per acre | |
| --- | --- | --- | --- | --- |
| | For maintenance lbs. | For production lbs. | For maintenance lbs. | For production lbs. |
| 1st Cutting | 62 | 40 | 2000 | 1300 |
| 2nd ,, | 60 | 36 | 2500 | 1530 |
| 3rd ,, | 57 | 33 | 2740 | 1610 |
| 4th ,, | 53 | 29 | 2700 | 1490 |

It will be noted that as growth progressed the crop became steadily less "concentrated," notably when appraised as food to be used for productive purposes. Thus, whereas 100 lbs. dry matter of the first cutting was equivalent for productive purposes to 40 lbs. starch, the same weight from the fourth cutting was equivalent to but 29 lbs. starch; or, in other words, 100 lbs. of the earliest cut hay was equivalent to 138 lbs. of the latest cut.

As regards the value of the total crop per acre, it will be seen that this reached its maximum at the third cutting, and although after this there was little depreciation in the maintenance value, there was a marked falling-off in the value for productive purposes. This deterioration is mainly due to the lowering of digestibility with advancing maturity, but there would also be mechanical losses of seeds in the latest stages owing to the ripeness of the plants. It should moreover be borne in mind that in the above estimates the pentosans have been classed as of equal value with the other carbohydrates. There is little doubt, however, that they are appreciably inferior in feeding value to starch and cellulose, and in view of the increase in the amount of pentosans recorded at the later cuttings the actual decrease in nutritive value of the crop must have been more marked than the above figures indicate.

## 1910 *Crop.*

The experiment was continued upon the same lines in 1910, except that at the third cutting duplicate plots were cut, in order to test the uniformity of the plots and the reliability of the methods of sampling, etc. As before, each plot was 20 sq. yds. in extent, and the whole of the produce was removed, dried under cover and weighed. After

reducing to fine chaff three samples were taken in each case, mixed together and this larger sample then analysed in triplicate.

The seeds were drilled on May 12, 1909, Standwell barley having been sown about a month earlier. No manurial dressing was given.

The cuttings of hay were taken on June 9, June 23, July 7, and July 21, 1910.

The character of the season is indicated by the appended meteorological data:

| Period | Rainfall inches | Sunshine hours | Average temp. °C. | Increase of crop lbs. dry matter per acre |
|---|---|---|---|---|
| April | 2·02 | 129 | 43° | |
| May | 2·09 | 177 | 51° | |
| June 1—9 | 0·26 | 48 | 55° | |
| ,, 9—23 | 0·52 | 112 | 59° | 1260 |
| June 23—July 7 | 3·89 | 49 | 50° | 120 |
| July 7—21 | 0·51 | 84 | 56° | 360 |

The outstanding feature of the weather was the dull, cold and almost continuously wet fortnight between June 23 and July 7, when under more genial conditions, as seen in the previous season, considerable growth would have taken place. It will be noted that up to this period the crop had been growing rapidly under genial conditions, but that during the spell of bad weather there was an almost entire cessation of growth, and this was not compensated in the succeeding fortnight, which was dry and sunny, with cool winds.

The weights of air-dried hay obtained at each cutting are given below:

| Date of cutting | Yield of Hay | |
|---|---|---|
| | Per plot | Per acre (to nearest cwt.) |
| | lbs. | cwts. |
| June 9 | 15¾ | 34 |
| ,, 23 | 21¼ | 46 |
| July 7 (Plot A) | 22 | 47 |
| ,, 7 (Plot B) | 21¾ | 47 |
| ,, 21 | 23¼ | 50 |

The condition of the crop at the times of cutting was roughly similar to that of the previous year's crop save for the little headway

made between the second and third cuttings. Furthermore in this season the leguminous plants were far less successfully established so that the hay was to a much greater extent a rye-grass hay.

It will be noted that two plots were cut on July 7 (third cutting), and practically identical weights of hay were obtained from each, the recorded difference being well within the limits of error of experiments of this character.

The composition of the crops is given in the appended table:

| . | 1st Cutting (June 9) | 2nd Cutting (June 23) | 3rd Cutting Plot A (July 7) | 3rd Cutting Plot B (July 7) | 4th Cutting (July 21) |
|---|---|---|---|---|---|
| | | | Percentage in air-dry hay | | |
| Total dry matter............ | 89·4 | 90·8 | 90·8 | 90·1 | 91·5 |
| | | | Percentage in dry matter | | |
| True protein ............... | 6·82 | 5·04 | 6·34 | 6·06 | 5·88 |
| " Amides " .................. | 1·76 | 1·99 | 1·15 | 1·33 | 0·69 |
| Ether extract .............. | 1·86 | 1·76 | 1·59 | 1·57 | 1·18 |
| Ash .......................... | 8·06 | 7·35 | 7·27 | 7·17 | 7·81 |
| Crude fibre ................. | 27·5 | 31·3 | 31·5 | 31·7 | 32·7 |
| Sol. Carbohydrates, etc.... | 54·0 | 52·6 | 52·2 | 52·2 | 51·7 |
| Pentosans ................. | 25·8 | 24·2 | 23·3 | 23·7 | 24·0 |
| | | | Percentages in ash | | |
| Including SiO₂ ........... | 21·9 | 26·0 | 36·5 | 36·1 | 37·3 |
| K₂O ........... | 28·3 | 23·2 | 21·2 | 19·7 | 21·8 |
| CaO ........... | 17·6 | 19·7 | 14·9 | 15·2 | 15·0 |
| P₂O₅ ........... | 8·2 | 5·8 | 5·5 | 4·9 | 5·9 |

The most striking feature of the composition of the hays throughout is the extraordinary poverty in protein, the dry matter containing but 5—6 per cent. as compared with 9—10 per cent. in the previous year's crop. This is only partly accounted for by the greater preponderance of rye-grass in the 1910 hay, the principal cause being undoubtedly the dull cool weather throughout the greater part of the period of growth. There is indeed abundant evidence from the earlier investigations of Wolff and others[1] that poverty in protein is a characteristic of fodder grown in such seasons.

The changes with advancing age were broadly similar to those observed in the preceding year, being the most clearly marked in the

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[1] *vide* Pott, *Handbuch der tierischen Ernährung*, I. 149.

case of "amides" and fibre, whilst the proportions of true protein and of pentosans showed but little change. The ash, though rather more constant in proportion than in 1909, again showed a steady enrichment in silica, especially up to the time of the third cutting, and was notably richer in this ingredient, in potash and in lime than the hay of the preceding year.

The following table gives the weights per acre of the various ingredients of the crop obtained at each cutting:

| | Pounds per acre | | | | |
|---|---|---|---|---|---|
| | 1st Cutting | 2nd Cutting | 3rd Cutting | | 4th Cutting |
| | | | Plot A | Plot B | |
| Total dry matter ......... | 3410 | 4670 | 4830 | 4740 | 5150 |
| True protein ............... | 235 | 235 | 305 | 290 | 305 |
| "Amides" ................. | 60 | 95 | 55 | 65 | 35 |
| Ether extract .............. | 65 | 80 | 75 | 75 | 60 |
| Crude fibre[1] .................. | 940 | 1460 | 1520 | 1520 | 1680 |
| Pentosans[1] .................. | 880 | 1130 | 1130 | 1130 | 1240 |
| Sol. Carbohydrates excluding pentosans ........ | 960 | 1320 | 1390 | 1320 | 1430 |
| Ash ....................... | 275 | 345 | 350 | 350 | 400 |
| $SiO_2$ ......................... | 60 | 89 | 128 | 121 | 150 |
| CaO .......................... | 46 | 66 | 52 | 52 | 60 |
| $P_2O_5$ ......................... | 22 | 20 | 19 | 17 | 24 |
| $K_2O$ ......................... | 78 | 80 | 75 | 67 | 88 |

The practical suspension of protein-formation by the plants during the period investigated is brought out very strikingly. Such little gain of nitrogenous substance as occurred between the first and second cuttings was wholly non-protein in character, and the subsequent changes amounted to little more than the conversion of this "amide" material into true protein.

In accordance with the character of the season the amount of crude fibre increased but slowly, especially after the second cutting, whilst the amount of carbohydrates increased at a rather slower rate.

In general the changes were similar in character to those recorded in the previous season, but were less pronounced after the second cutting, the only appreciable increases then being in crude fibre and silica.

[1] See footnote to table, p. 310.

*Digestibility and Nutritive Value of Hay.*

The following table gives the data for the digestibility and relative nutritive values of the hays obtained at the different cuttings, these data having been arrived at on the lines explained in connection with the 1909 crop (p. 310).

*Digestible ingredients per cent. of dry matter.*

|  | 1st Cutting | 2nd Cutting | 3rd Cutting | | 4th Cutting |
|  |  |  | Plot A | Plot B |  |
|---|---|---|---|---|---|
|  | per cent. | per cent. | per cent. | per cent. | per cent. |
| True protein | 5·42 | 4·08 | 4·38 | 4·12 | 3·36 |
| "Amides" | 1·76 | 1·99 | 1·15 | 1·33 | 0·69 |
| Sol. Carbohydrates + Fibre | 54·0 | 52·6 | 52·2 | 52·2 | 51·7 |
| Ether extract | 0·9 | 0·9 | 0·8 | 0·8 | 0·6 |
|  | lbs. | lbs. | lbs. | lbs. | lbs. |
| Starch equivalent of 100 lbs. dry matter: |  |  |  |  |  |
| For maintenance ... | 63½ | 60½ | 60 | 59½ | 57½ |
| For production ...... | 44 | 39 | 38½ | 38½ | 36½ |

*Digestible ingredients per acre.*

|  | 1st Cutting | 2nd Cutting | 3rd Cutting | | 4th Cutting |
|  |  |  | Plot A | Plot B |  |
|---|---|---|---|---|---|
|  | lbs. | lbs. | lbs. | lbs. | lbs. |
| True protein | 174 | 173 | 195 | 176 | 158 |
| "Amides" | 60 | 95 | 55 | 65 | 35 |
| Sol. Carbohydrates + Fibre | 1840 | 2460 | 2520 | 2470 | 2660 |
| Ether extract | 32 | 41 | 39 | 38 | 30 |
| Starch equivalent: |  |  |  |  |  |
| For maintenance ...... | 2160 | 2820 | 2900 | 2820 | 2960 |
| For production ........ | 1500 | 1820 | 1860 | 1830 | 1880 |

The results in the main are similar to those obtained with the 1909 crop, the percentage of digestible protein diminishing appreciably as growth was prolonged, and the whole fodder becoming less "concentrated," as shown by the steadily falling percentage starch value. There is no evidence, however, of any diminution in the nutritive value of the total produce per acre. Still it must be remembered that the produce from the experimental plots was so dealt with as to avoid any mechanical

losses. The plants were, however, so ripe at the time of the fourth cutting that there would inevitably have been considerable mechanical losses of seeds, etc., in winning the crop by ordinary methods, and consequently an appreciable diminution in the value of the crop. It is probable, moreover, that the greater quantity and coarser texture of the fibre in the hay obtained at the last cutting have not been sufficiently allowed for in calculating the starch-values. We are, therefore, of opinion that the third cutting, early in July, as in the previous year represented the most valuable crop. The crop certainly did not appreciate in value after this date.

## SUMMARY.

The study of two years' hay crops indicates that the composition of the hay steadily changes throughout the period commonly covered by the hay-making season.

The nature of the change depends to some extent upon the character of the season. In the summer of 1909 when the climatic conditions favoured steady growth there was a steady production of protein and fibre and a considerable assimilation of ash ingredients, notably silica. In the following summer the effects of a severe climatic set-back in the later part of June was seen in an almost complete suspension of growth, and notably in the production of protein.

In both seasons there was a steady fall all round in the digestibility of the hay, which in the end more than counterbalanced the increase in weight of the crop.

In both seasons the best results were obtained by cutting about the beginning of July. A fair amount of latitude in the time of cutting —say, a week or 10 days—may be allowed, however, without very seriously impairing the nutritive value of the crop. There was evidence, nevertheless, in each year of appreciable deterioration after the middle of July.

# THE PHOSPHATE NUTRITION OF PLANTS.

By ALLAN BAGULEY, B.Sc., F.I.C.

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MUCH work has been done in America, on the Continent and in this country, to determine the effect of different finely-ground naturally-occurring phosphatic substances on plants of various natural orders, grown under differing conditions as to the character of the soil and the use of other nutrient substances. In a few cases results or conclusions appear to conflict. Not nearly so much attention has been devoted to the effect of artificially prepared insoluble salts of phosphoric acid on the growth of plants. When these are employed in the absence of ammonium salts, and acid soil substances, definite information should be obtained as to the powers possessed by plants themselves of utilizing insoluble phosphates. With the object of making a contribution to such knowledge, oats, peas and swede turnips were grown in artificial soils made up of white sand and pure chalk, and the particular salt of phosphoric acid under investigation. The other essential nutrient substances were supplied in a dilute solution of salts.

The phosphates employed were normal ortho-phosphates of calcium, iron and aluminium. They were prepared according to the method described by Warington[1].

Tri-calcium phosphate was obtained in the following way. The tri-sodium salt was first of all prepared by adding an equivalent of sodium hydroxide to di-sodium hydrogen phosphate. The solution diluted to normal strength was treated with an equal volume of a semi-normal solution of calcium chloride slowly run in with constant stirring. The precipitate washed repeatedly by decantation till free of chlorides was finally collected in a Buchner funnel and dried at a temperature of about 60° C. The product was next subjected to analysis. 0·3981 gram was dissolved in dilute hydrochloric acid, reprecipitated with ammonium hydroxide, and the precipitate redissolved in

[1] Warington, *Journ. Chem. Soc.* 1873, p. 984.

acetic, acid. The calcium was precipitated by ammonium oxalate in the acetic acid solution, and the phosphoric acid in the filtrate by magnesia mixture.

The results obtained were:

|  | Found | Calculated for $Ca_3(PO_4)_2$ |
|---|---|---|
| CaO $°/_o$ ...... | 54·22 | 54·19 |
| $P_2O_5$ $°/_o$ ...... | 45·78 | 45·81 |

The substance was evidently $Ca_3(PO_4)_2$ in a high state of purity.

Normal ferric ortho-phosphate was prepared by slowly running, with continual stirring, a semi-normal solution of ferric chloride (previously oxidised by treatment with chlorine in sunlight) into an equal volume of a normal solution of di-sodium hydrogen phosphate. Iron was estimated by titration with potassium permanganate, phosphoric acid by precipitation with ammonium molybdate, and water by ignition.

The following results were obtained:

|  | Found | Calculated for $Fe_2O_3 . P_2O_5 . 4H_2O$ |
|---|---|---|
| $Fe_2O_3$ $°/_o$ ...... | 42·46 | 42·78 |
| $P_2O_5$ $°/_o$ ...... | 37·85 | 37·97 |
| $H_2O$ $°/_o$ ...... | 19·34 | 19·25 |

The substance was evidently ferric phosphate practically pure.

Aluminium phosphate was prepared by the slow addition with constant agitation of a semi-normal solution of aluminium sulphate to an equal volume of a normal solution of di-sodium hydrogen phosphate. The precipitate was treated in the same way as the others and was found to contain 48·36 per cent. $P_2O_5$. The different phosphates were ground sufficiently finely to pass through a sieve having 45 meshes per inch. When more finely ground it was found that subsequent operations were rendered very difficult. The next process was ignition, carried out in the case of the ferric phosphate at a lower temperature to avoid the fusion which would otherwise occur. To obviate the reducing effect of the flame, the ignition was performed in an iron vessel surrounded with a graphite bath. The ignited residues were then extracted with successive large quantities of boiling water, until no further decomposition took place.

The extracts were found to be acid in reaction, and, owing to the large mass of phosphate, the process was prolonged. The nutrient salt solution was found to have no effect on the phosphatic residues at the ordinary temperature.

An attempt was made to prepare a trimetallic magnesium phosphate

in the same way as the calcium salt. The product however was found to undergo simple solution in hot water. There was no prospect of obtaining an insoluble basic residue as in the other cases and, as it was notably soluble in cold water, work with magnesium phosphate was abandoned.

The medium in which the plants were grown was chiefly made up of pure white sand freed from dust, which had been extracted with hot concentrated hydrochloric acid, washed and dried, and ignited. This was mixed with 3 per cent. of pure calcium carbonate and 0·5 per cent. of ferric hydroxide, and the particular prepared phosphate in such a quantity as to contribute 0·25 per cent. of $P_2O_5$ to the whole mass. These components were carefully mixed, each ingredient being weighed out for each vessel separately so as to secure uniformity of composition. Common flowerpots of two sizes were employed as containing vessels, the smaller size being filled with 1800 grams of the mixture, the larger size requiring 4200 grams. Previous to use the flowerpots were digested for several hours in dilute hydrochloric acid, thoroughly washed with water and dried. They were next painted internally with paraffin wax. Deep saucers were used to catch the drainings from the pots and thus conserve and return the nutrient salts. The plants were watered sometimes through the pots and sometimes through the saucers. Controls were afforded by pots containing 0·1 per cent. of soluble phosphate in the form of commercial superphosphate of lime and by pots containing no phosphate at all. The nutrient solution contained 5 grams $KNO_3$, 3 grams $MgSO_4 . 7H_2O$ and 1·5 grams NaCl per litre. The large pots received in all 200 c.c. of nutrient solution and 0·2 gram $FeSO_4 . 7H_2O$ and the small pots each received half these quantities.

Peas and oats were sown, three seeds in each pot. Turnip seed was used freely and the plants thinned out while very young.

Grown with ferric phosphate oats made strong, very well-developed plants with dark foliage, and plenty of seed ripening late: peas made healthy sturdy plants with well-developed pods not very numerous, ripening late: swedes grew steadily and well from the start, finally making very good plants.

Grown with aluminium phosphate oats became strong well-developed plants, dark in colour, ripening late: peas grew into healthy sturdy plants with well-developed pods not very numerous, ripening late: swedes grew steadily and well from the start, finally making good plants.

Grown with calcium phosphate, oats germinated and died: peas

made poorly developed plants with few flowers and puny pods ripening early: swedes commenced to grow about six weeks late and then continued to grow normally becoming healthy plants though backward.

On the whole the effect of ferric phosphate was very similar to that of aluminium phosphate. Compared with the superphosphate plant, the oats did not tiller so well as these latter but, on the other hand, they were in each case, clearly stronger in the straw and longer in the ear, being finer plants altogether. The swedes were both larger and more healthy than with the superphosphate. The peas grown with ferric phosphate, and with aluminium phosphate, were neither so early nor so fruitful as when grown with superphosphate.
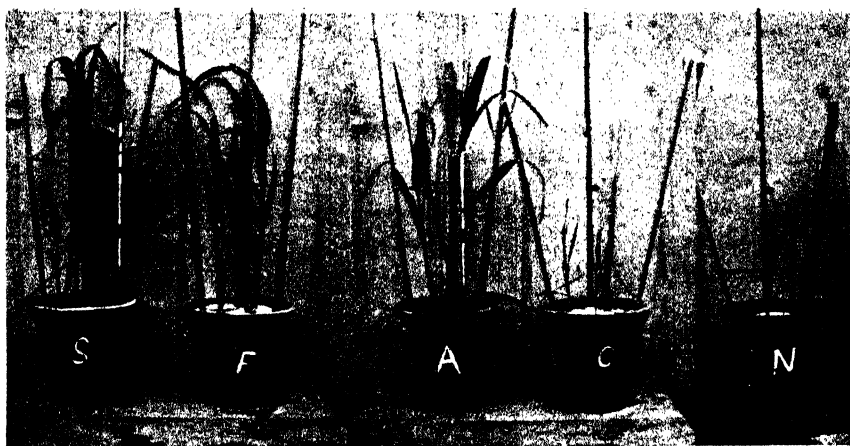


The photograph shows the oat plants about half grown. The initial on the pot indicates the phosphate used in each case. *S* denotes superphosphate and *N* no phosphate.

With calcium phosphate the results were very different. The oat plants did not thrive any better than those grown entirely without phosphate, being very ill-developed and producing no ear at all. Until about six weeks after sowing the same appeared to be true of swedes, but then, when the plants nourished with iron phosphate and with aluminium phosphate were well started, they too began to grow vigorously, and at the end of the season they were good healthy plants though by no means so forward as the others. The peas grew moderately well, but the plants ripened early and the crop was poor.

Subsequent experiments were carried out with the object of ascertaining the effect of ignition and extraction with boiling water on the availability of the precipitated phosphates.   Aluminium phosphate was found to be so generally similar in its effects to ferric phosphate that it was not included in these trials.   The phosphates of iron and calcium were prepared and ground as before but portions were differently treated. The results are set out in the table :

| | Super-phos-phate | Ferric Phosphate | | | Calcium Phosphate | | | No Phos-phate |
|---|---|---|---|---|---|---|---|---|
| | | Unignited Extracted | Ignited Unextracted | Ignited Extracted | Unignited Extracted | Ignited Unextracted | Ignited Extracted | |
| Oats ... | 2294 | 724 | 517 | 347 | 336 | 177 | 170 | 206 |
| Swedes | 1252 | 220 | 167 | 103 | 263 | 250 | 146 | 7 |
| Peas ... | 1873 | 1886 | 1601 | 1129 | 1560 | 1089 | 1100 | 923 |

The figures represent milligrams of dry matter per plant.   The plants were taken when only partly grown.

The figures show (1) that ignition and extraction with boiling water exercise a considerable effect on the availability of the phosphate, (2) that insoluble calcium phosphate is markedly less effective in the growth of oats than is insoluble iron phosphate, (3) that this difference in efficiency is not apparent in the case of swedes and peas.

The results as a whole are in accord with the conclusions of Söderbaum (*Bied. Centr.* 1902, XXXI. 203, from *Med. kongl. Landtb.-Akad., Exper. Stockholm*, No. 67, 1—15), who worked with an essentially normal tri-calcic phosphate, prepared electrolytically from apatite.   He conducted pot experiments with oats and found that not much phosphoric acid is available for the oat plant.   Prianischnikoff (*Landw. Versuchs. Stat.* 1906, LXV. 23) also finds that even in pure sand lupins can still utilise apatite though Gramineae fail to do so.

# BACTERIAL DISEASES OF PLANTS[1].

By Professor M. C. POTTER, Sc.D., M.A.

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In discussing among men of science the subject of diseases caused by bacteria, it is quite unnecessary, at this date, to argue the question as to whether there are or are not bacterial diseases of plants. A knowledge of the literature of the past fifteen years and of the character of the researches which have been carried out in this branch of phytopathology, establishes the point beyond all doubt. But it may be useful to consider briefly some of the reasons which accounted for the scepticism prevalent in earlier days.

The acceptance of a new truth is always difficult, especially when there is prejudice to overcome, and unfortunately in this case there existed widespread misconception of the nature of the problem under consideration. It might have been supposed that the brilliant work of De Bary, which showed the entrance of the germ-tube of a parasitic fungus into the host, and thus laid the foundation of our later knowledge of both animal and plant pathology, would have prepared the way for a belief in the parasitic invasion of plants by bacteria. But De Bary himself, among others of the older botanists, held the view that the acid secretion of the cell-sap presented an unfavourable medium for the growth of bacteria, and it was often maintained, notably by Hartig, that the absence of channels suited to their distribution and the impervious character of the cell-wall precluded the possibility of bacterial intrusion. It was thus the fashion, until quite recent years, to regard any damage caused by bacteria in plant tissues as being of saprophytic origin only.

These ideas gradually gave way under the accumulating mass of evidence which proved under rigid conditions of experiment that

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[1] Paper read at the Meeting of the British Association at Portsmouth, on September 1st, 1911, to introduce a discussion upon Bacterial Diseases of Plants.

bacterial diseases of plants could be reproduced in healthy tissues with absolute certainty of effect, and that certain virulent types could be communicated very rapidly and develop advanced symptoms of the characteristic disease in a remarkably short time and as surely as any of the most virulent forms of animal parasites.

It has been shown that vegetable tissues are not uniformly acid, the sap may be either acid or alkaline, and these two reactions may be found in different parts of the same plant. Also the acidity of the cell-sap by no means prevents the growth of bacteria, there are in fact certain species which find their most favourable development in an acid medium and many others possess the power of neutralizing the acid and converting it into an alkaline medium suitable for their growth.

The power of adaptability of bacteria to unfavourable conditions is remarkable, and the cultural variations which represent the medium most adapted to the needs of the different organisms make it unsafe to assume on *a priori* grounds that any conditions must necessarily be prohibitive to bacterial life. Some bacteria will only grow within very narrow limits of temperature, others enjoy a wide range ; certain of the thermophile species will thrive best at temperatures above 50° or 60° C. and are not destroyed with a heat approaching 70° or even 75° C., while others flourish at a few degrees above the freezing point. *Bacillus subtilis* will only grow in the presence of free oxygen, other species only in the absence of oxygen. Strong malic acid promotes the growth of *B. amylovorus* while altogether inhibitory to that of most other bacteria. Many, under special conditions of the nutrient media, will flourish in the presence of antiseptics such as chloroform, phenol, thymol, and even corrosive sublimate in percentages usually supposed to be quite inhibitory, and some forms have been known to enjoy existence in a solution quite blue with copper sulphate. Thus, though plants may possess many bactericidal properties, these may prove quite ineffective in certain cases, and the wonderful vitality of some such micro-organisms and their wide divergence as regards physiological requirements opens out a broad basis for their operations.

The plant possesses many vulnerable points of attack, and infection has been shown to take place through the water-pores, the stomata, the floral nectaries, and through wounds, the vessels of the xylem affording a ready means of dispersion for the invading organism. Further, the researches of Laurent and myself, and later of several other investigators, have disposed of the "impassable barrier" supposed to be offered by the cellulose membrane to the entrance of bacteria. It has been proved

that certain species are able to secrete both a toxin, which plasmolyses and kills the protoplasm, and a cytolytic enzyme which causes the dissolution of the middle lamella and the softening and swelling of the cell-wall. After the toxin has effected the destruction of the protoplasm and the cell-membrane has become very much softened by the cytase, the bacteria have the power of perforating the walls and passing into the cell-cavity, thus working their way deeply into the tissues of the plant.

In this brief paper it is not possible to consider morphological and cultural characters of the parasites, and I propose to give only a general description of the pathogenic properties of some of the more common typical forms, leaving a complete account of special diseases to those who follow.

There are various well-marked types of bacterial disease which differ considerably in their pathological character and in the extent of the injury which is produced, though it is difficult to separate them under any definite system of classification. A very large number of highly destructive diseases fall under the head of the " Soft Rots," a form of parasitism in which there is rapid degeneration of the cell-wall and complete destruction of the parenchymatous tissues. The bacterium causing the white rot of turnips, which I have specially investigated, illustrates a very virulent form of parasite and may be taken as typical of the pathogenic action of this class of " Rot."

This organism I described as belonging to the genus *Pseudomonas*, though perhaps it is more truly a *Bacillus*. It was isolated from turnips attacked in the field and inoculations from pure cultures upon perfectly sound bulbs will reproduce with great certainty all the characteristic effects of the white rot in about twelve hours, the decay spreading from the point of infection through the root until at the end of only a few days the greater part of the flesh becomes of a greyish pearly-white colour, quite soft to the touch, and eventually it is reduced to a white putrefying mass of rottenness.

Both when grown in a nutrient solution and on a living turnip this bacterium was found to secrete an enzyme which has a very marked action in causing the dissolution of the middle lamella and the softening and swelling of the cell-wall. It also secretes a toxin which acts in plasmolysing and killing the protoplasm. A section of the root inoculated with a pure culture of the bacterium shows a complete dissociation of the cell-tissues, the bacilli crowd along the intercellular spaces and the middle lamella and occupy the cell-cavity, the protoplasm is destroyed,

the cell-walls lose all rigidity, becoming much swollen and striated and so soft that great difficulty is experienced in manipulating the preparations. The enzymic activity is very rapid as is shown by the fact that the walls have been found to swell from $2 \mu$ to $7 \mu$ in the course of 24 hours. With the death of the protoplasm the cell-sap escapes into the intercellular spaces, and additional nutriment is thus afforded for the bacteria which continue to multiply with enormous rapidity and set free both cytase and toxin, and in this manner the process of disorganization proceeds throughout the tissues. The parasite can readily effect an entrance into its host through the undeveloped epidermis of young and tender structures, but the hard and tough rind of the sound turnip is entered only through a wound where the injured cells afford it sufficient nutrition and energy to make the initial attack.

Though differing in some particulars, the bacterial action here described is in the main characteristic of most of the soft rots which attack a considerable number of common vegetables and other plants. *P. destructans*, however, appears to possess the greater power of virulence, as it suffers no reduction in pathogenicity through long cultivation, and there is certainly invasion of the cell-cavities by the bacilli, which is stated not to occur in the other cases recorded. The causal organism has been described by Van Hall for an attack of *Iris florentina*, by Jones for a rot of carrots and other allied plants, and by Harrison for a disease of the cauliflower, and in each of these cases an enzyme has been isolated. Jones, Harding, and Morse have shown that all these three parasites must be considered to be truly representative of but one species, *Bacillus carotovorus*, and they conclude from an interesting comparative study of innumerable pathogenic varieties that the whole of the soft rots caused by a non-chromogenic liquefying bacillus represent a group of very closely related organisms.

Another group of bacterial diseases may be conveniently classified as the " Black Rots," the special feature being the presence of dark brown or black patches in the stem and leaves, especially in and around the xylem vessels. There may be subsequent invasion of the parenchymatous tissues by the bacteria but the action is comparatively slow, the rapid swelling of the cell-wall is not a conspicuous feature, and the tissues do not become broken down into a soft mass. The " Black Rot of the Cruciferæ " (*Pseudomonas campestris*), a disease widely spread in Europe and North America, may be mentioned as a typical example. Many roots are found with the peripheral vascular bundles marked out as a dark ring and large black patches extending to the centre. This attack

in the early stages is confined to the vascular bundles where the vessels are crowded with the bacteria. The rot extends along the medullary rays, thus creating a characteristically radial structure, bacterial cavities are formed by slow solution of the surrounding cell-walls, and the root is often left with a central hollow in which alternating portions of the woody strands alone persist. In a form which I have found to attack the full-grown root, the vessels are blocked with a kind of gummy substance, and as the disease progresses this " gum " extends into the intercellular spaces and later into the cell-cavities, until finally the entire bulb becomes discoloured; but externally the root may appear quite sound, the disease producing a kind of dry rot internally.

Very similar in many respects to the above, and difficult to separate in their pathological aspects, are other diseases which may be more particularly considered as the " Bundle Rots," in which again the xylem vessels are primarily attacked and become filled with numerous bacteria. It is well known that the xylem vessels contain soluble carbohydrates and other substances which constitute a favourable medium for the growth of bacteria, and infections take place readily through the water-pores and glands which are continuous with the termination of a fibro-vascular bundle. The bacteria thus find an easily accessible channel for their progress and are enabled to multiply and work their way along the wood vessels and so invade the entire plant. The vessels become so blocked with bacteria that the supply of water is cut off, and one of the primary symptoms of these diseases is the wilting of the foliage due to the arrest of the transpiration current.

The potato and tomato disease (*B. solanacearum*, Smith) may be cited as an example. This attack first makes its appearance by the sudden wilting of the foliage, often at first on a single shoot, but subsequently the entire plant is affected. The stem shrivels, changing from green to brown or black. The vascular bundles, even in the early stages, can be detected as black lines showing through the cortical tissues. Still by means of the xylem vessels, the bacteria travel through the stem into the potato tubers, where the same appearance of the blackened vascular bundles is evident. Here again the vascular system is traversed first, and only in advanced stages does the starch-bearing parenchyma become affected. Eventually, however, the organism attacks and destroys the parenchyma, converting this tissue into a watery mass of broken down cells.

Of a somewhat similar nature are the diseases known as " Schwarz-beinigkeit " or " Black-leg " (Appel); the " Black Stalk-rot " (Pethybridge);

the rot caused by *B. solanisaprus* (Harrison); and the "Tabakwelk-krankheit" (*B. nicotianæ*, Uyeda).

The wilting of various Cucurbitaceæ, which has been described by Smith as caused by the *Bacillus tracheiphilus*, strikingly manifest another form of this type of disease. Here the bacilli more particularly occupy the spiral vessels and later the tracheides, and the most marked symptom is the wilting of the leaves, though later the bacilli pass onwards through the vascular tissue to the stem and eventually all the internal tissues become more or less destroyed.

Among other bacterial diseases which are restricted more peculiarly to the vascular tissue should be mentioned the classic example of the yellow disease of hyacinths (*Pseudomonas hyacinthi*), the ætiology of which was so admirably worked out by Wakker as early as 1881. The parasitic action is extremely slow and any invasion of the acid parenchymatous tissues very difficult. The bacteria extend along the vessels which become filled with a thick viscid yellow mucilage, and in advanced stages the walls of the xylem may be dissolved by this yellow slime and continuous tubular cavities become formed. The first symptoms of the disease are noted in the foliar and floral organs. There is brown striation of the leaves and the epidermis frequently ruptures longitudinally and exudes an abundance of bacterial slime. Eventually, though only after many months, the bulbs become diseased and a great part of the scales may degenerate into a mass of yellow slime. The sticky slime which oozes from the affected leaves is highly infectious, it adheres to anything it touches, and the germ may thus be readily carried from one plant to another in ordinary gardening operations.

The disease of sweet corn, a variety of *Zea mais*, described by F. C. Stewart, is one in which the organism is confined exclusively to the vascular system. It may occupy the vascular tissue throughout the entire plant, but is found only in the fibro-vascular bundles, the cells of the parenchyma are never invaded, and there is no disorganization or discolouration of any of the tissues. The vessels form the chief stronghold of the bacteria and they exist in these channels in such numbers that they are described as issuing out of the ends of the vessels "like smoke out of a chimney." This disease has not been definitely established by a pure culture inoculation, but there seems no doubt as to its pathogenic nature.

That all the diseases previously mentioned are due to bacteria has been established beyond all doubt, by the most careful cultural experiments and by repeated inoculation with pure cultures. Much, however,

still remains to be done respecting the physiology of the organisms. The existence of enzymes and toxins has not yet been determined in each case, nor the changes taking place in the cell-walls, and the origin and nature of the gummy substance, which is so often an accompanying phenomenon, remain to be investigated.

I may here just briefly allude to a disease of the orchid, *Odonto-glossum Uro-Skinneri*, which I have been investigating and which exhibits some rather special features. The symptoms are black blotches upon the leaves where the epidermis is raised like a blister, and the presence of a large amount of gummy substance in the intercellular spaces, particularly in the respiratory cavities of the stomata. Another characteristic is the destruction of the chlorophyll corpuscles, which in the early stages enlarge as if vacuolated by the decrease of the isotonic coefficient of the cell, and as the disease progresses they finally disappear. This disease appears almost certainly to be due to a bacterium, though up to the present time inoculations with bacteria obtained from the blotches have only produced negative results. Bacteria, however, have been detected in the attacked areas by means of special preparations and staining.

The tumorous diseases, which are found upon a great variety of plants, constitute a special class quite different in type from those already considered. Several of these have been traced to a bacterial origin.

The tuberculosis (rogna) of the vine and the tumour of the olive have been definitely traced to bacteria by isolation of the true causal organism and successful inoculations by means of pure cultures therefrom, and it seems probable that the tumours of the pine and other trees are also truly ascribed to the action of Schizomycetes.

Lately E. F. Smith has greatly extended our knowledge of these tumours in his deeply interesting work upon the Crown Gall. After a prolonged study Smith and his colleagues isolated a white bacterium (*Bacterium tumefaciens*) from the galls upon *Chrysanthemum frutescens*, which produced, by pure culture inoculations, similar galls upon this plant and also upon various species of Compositæ, Oleaceæ, Vitaceæ, Leguminosæ, Cruciferæ, Umbelliferæ, Solanaceæ, and Chenopodiaceæ. Innumerable successful cross-inoculations have been made between plants of these orders, and a bacterium closely resembling *B. tumefaciens* has been isolated from natural galls on many different hosts which again induced the tumours on the daisy and various other plants.

The Crown Gall disease is one which progresses very slowly, the size

and rate of growth of the galls depending upon the state of nutrition and activity of growth of the plant. Old and slow growing tissues are not favourable to its development. The organism has not been stained *in situ* but it is believed to occupy the living cells in small numbers and by its presence stimulates to rapid cell-division, thus producing eventually the galls or tumour-like structures. The excessive enlargement of individual cells of the hyperplasia, such as is characteristic of the galls of *Plasmodiophora* and of those formed by *Azobacter* and *Urophlyctis* on the Leguminosæ, is not found in these tumours. The cells in the proliferated mass, on the contrary, are often smaller than those of the normal tissues, the stimulus to division being too active to permit of normal development.

The tumours always arise in one or other of the meristematic tissues, the cells of which continue to divide without any physiological limitation, producing an exuberance of parenchyma and a corresponding reduction in conducting tissue. The nature of the tumour varies with the meristematic tissue from which it is derived. Some are characterized by preponderant masses of proliferating parenchymatous tissue permeated with contorted vascular bundles more or less lignified; while others are hard and woody, their structure consisting chiefly of twisted and contorted lignified vascular bundles and abundant woody fibres mingled with some parenchyma.

Of special interest is the tendency to produce secondary tumours at some distance from the point of inoculation or from another primary tumour; the bacteria must thus travel in some way along the plant tissues but the actual method of migration has not been determined. This recalls the wandering of morbid growths in animal tissues, and the author of this investigation discusses very suggestively the resemblances existing between the phenomena exhibited by these plant tumours and malignant animal tumours such as Sarcomata. The comparison is very striking and of the utmost physiological and pathological importance.

Nothing can more clearly exemplify the difficulty of running a bacterial disease to earth than this brilliant piece of work from the Laboratory of Plant Pathology at Washington, and it shows that the failure to isolate a bacterium and reproduce the disease, in cases showing suspected bacterial action, does not exclude the possibility of its being due to this cause. Hundreds of inoculations, made with bacteria obtained from the interior of the galls, during a continuous study extending over two years, failed to secure any positive result, stains did not succeed in discovering the bacteria in the tissues, and the cause of the disease

remained a mystery. Then it became known that the tissues contained a number of saprophytic organisms, which developed in the cultures much more rapidly than the true parasite, and the negative inoculations were most probably all made with these saprophytes. Inoculations made from the small white colonies which appeared after five days and were obtained from the deeper tissues, produced the characteristic galls and thenceforth they could be developed with certainty in a great variety of plants and convincing proof was given of the bacterial nature of this disease.

Some confusion exists as to the nature of the diseases included under the head of "Cankers." Some of the so-called cankers are due to the work of insects, but the true canker growths are undoubtedly of bacterial or fungoid origin. Lately considerable doubt has been thrown upon the conception that the cankers of the pear, apple, and ash are due to *Nectria*. Though it has been claimed that these cankers have been produced by pure inoculations of *Nectria ditissima*, there is always the danger, not sufficiently guarded against, that bacteria have been introduced with the cultures of the fungus. Noack, perhaps without sufficient evidence, has ascribed the ash canker to bacteria, but the work of Brzeziński upon the canker of the apple and pear is based upon surer ground. Brzeziński has isolated in pure culture two bacteria (*Bacterium pyri* and *Bacterium mali*) and these when inoculated upon the apple and pear again produce the typical cankers. He has also found that tumours can be developed by these bacteria. Although Brzeziński's conclusions have been strongly criticized, especially by Aderhold, it seems probable that the *Nectria* is after all only a saprophyte living upon the tissues destroyed by the bacteria, and this is borne out by the fact that true cankers are known in which no hyphæ can be detected.

Before leaving the tree diseases, one must briefly mention the well-known "Fire-blight," so called from the appearance presented of scorching by fire or frost, which was described by Burrill as early as 1881 as due to *Bacillus amylovorus*. The bacteria infect the flowers through the nectaries, where they are able to live and multiply and thus are carried by insect visitors from flower to flower and from one tree to another, until a veritable epidemic is produced. The parasite may also effect an entrance through the tips of the growing twigs, it attacks the cortex and cambial layers and the blight extends through the branches and often to the main trunk. The collapse of the foliage is a striking feature, the leaves hanging dead and brown from the tree,

and dead areas forming cankers may also be observed on the affected trunks. The "Fire-blight" is exceedingly destructive in Canada and the United States, but fortunately so far it is unknown in Europe.

As far as investigations upon the diseases of plants which are due to bacteria have been pursued, they have established a complete homology with those diseases which are caused by fungi. In both cases it is recognised that environment and the everchanging external conditions play a most important part, and the health of the host and parasite are alike affected by physiological considerations underlying special features of cultural treatment. The direct relation of the character of the cell contents to the question of immunity is well ascertained and the phenomena of positive and negative chemotropism are often determining factors. A very intimate relationship exists between the chemical composition of plants, their food supplies, and the development of their parasites.

As we have seen the sap of plants may be either acid or alkaline, and according to the character of the food material supplied the excretion of certain products of metabolism may create either an acidity or alkalinity in the surrounding medium.

In this connection it is important to remember that many bacteria which are normally saprophytic are capable of becoming actively pathogenic in plant tissues, when they find conditions peculiarly adapted to their growth, and an unhealthy state of the plant renders it less resistant to attack. Possibly finding in injured cells a nutrition on which they flourish with great vigour as saprophytes, the bacilli may then become gradually educated to a parasitic habit until they end by acquiring a high degree of virulence. Laurent has shown that two common species, the *Bacillus coli communis* and *B. fluorescens putidus*, normally incapable of attacking plants, could be rendered very actively parasitic upon the potato and other tuberous plants after they had first been deprived of resistance by special cultures in alkaline media. Successive cultivations upon tubers of slight resistance produced eventually a surprising degree of virulence. Lepoutre extended these investigations to three other species: *Bacillus fluorescens liquefaciens, B. mycoides,* and *B. mesentericus,* and obtained precisely similar results, the parasitic aptitude of *B. fluorescens* being especially remarkable and proving it to be a very dangerous enemy of many cultivated plants. The experiments of Lepoutre entirely confirmed Laurent's conclusions that chemical manures have a strong influence upon the resistance of tuberous plants to bacterial infection, and that an excess of nitrogenous manure or of lime

predisposed to rot while phosphates increase the resistance of turnip and carrots to virulent bacteria. The products of the decomposition of nitrogenous materials in the cells induces alkalinity. As Lepoutre has pointed out the action of bacteria in decomposing the sugars produces organic acids, which added to those acids already present in the cell-sap would have a toxic effect upon the bacteria. The intervention of ammonia is necessary to hinder this action and this explains the danger of employing nitrogenous manures in quantity. Such a food provokes a greater assimilation of nitrogenous combinations, all very favourable to the nutrition of bacteria, and the production of ammoniacal residuary compounds.

With regard to the sugar beet there is evidence that excessive nitrogenous manuring prevents the accumulation of sugar, delays the ripening, and also accelerates decay during storage. Such treatment affects the potato in a similar way, it prevents the storage of starch and renders the plants especially liable to the Black dry-rot (Schwarze Trockenfäule) and Eisenfleckigkeit or Buntwerden and the tubers less resistant to decay during storage in the winter months. On the other hand potash and phosphatic manures enable the potato more readily to withstand these diseases. Again, nitrogen in excess produces similar effects upon the cereals and also renders many greenhouse plants liable to suffer from such fungi as *Botrytis* during the winter months.

In the case of fruit and many bulbs it is the percentage of sugar which determines whether or no *Penicillium* can become a parasite and the constant variation in the sugar content and the amount of oxalic acid present are circumstances also largely affecting bacterial infection. The gradual change in the reserves of roots and tubers is worth studying in its bearing upon this point. It is a common but quite erroneous statement that the turnip and swede are devoid of starch. In the first period of active growth, marked by the accumulation of reserve materials, starch is an abundant product, and its presence can readily be detected in these " roots " during autumn and early winter. As time proceeds the starch is gradually replaced, first by cane sugar and subsequently by grape sugar. The relative amounts of these three carbohydrates vary during the dormant period. In October and November all three may be present, while in the early spring only the two sugars are to be found. This latter period coincides with that of the greatest content of grape sugar, the roots then become " soft " and specially liable to attack and different phases of immunity accompany the differences in the relative amounts of carbohydrates.

The changes in the reserve materials of the potato again react upon its bacterial parasites, and the immunity preserved in certain stages of growth may be explained by the changes in the chemical composition of the tuber. It is well known that varieties which are richer in protein are more liable to bacterial rot, while those richer in starch are more resistant. The nature of the non-nitrogenous reserves, however, is materially influenced by changes of temperature, and when the tubers are exposed to slight frosts there is conversion of starch into sugar and they then become more susceptible to bacterial invasion.

All these facts must be taken into account when we seek to explain how plants come to be attacked and entirely succumb when quite mature and apparently in full vigour.

In addition to the amount and nature of the food supplies, we must consider under environment which materially influences the susceptibility to disease both as regards the host and parasite, the variations of light, temperature, and vapour pressure of the air, the temperature of the soil, and the circulation of both water and air in the soil.

It is well understood that atmospheric conditions largely affect bacterial diseases. Sunlight is the most powerful bactericidal agent known, and at the same time under full illumination the normal metabolic processes of the plant are at their optimum. On the other hand, damp foggy weather not only promotes bacterial growth but hinders the vital activity of the plant-cells, rendering them less able to resist attack.

The important part played by free oxygen in the soil is hardly sufficiently recognised, and that any circumstances which prevent the circulation of air in the soil have a most prejudicial effect. For effective functional activity of the roots energy is required, and this energy is supplied by respiration. Respiration involves the evolution of carbon dioxide, a gas which has a pernicious effect when present in excess, and conditions in which it is allowed to accumulate lead to profound changes in the metabolic products of the cell. The deprivation of oxygen induces intra-molecular respiration with the formation of alcohol and hence alcoholic and acetic fermentation.

Circumstances such as the water-logging of the soil—or the surface crustation—to which certain soils are specially liable after rain or watering, prevent the free circulation of the air. The roots are thus deprived of oxygen by the accumulation of carbon dioxide in the soil, transpiration is diminished and also the cell turgor. The unhealthy conditions induced by this oxygen starvation render the plant specially

liable to fall a prey to disease. A good example of the predisposition to bacterial diseases through such causes is given by the Root-rot of the sugar beet and mangel. The unfavourable soil conditions so alter the constitution of the root-cells that the plant is rendered readily susceptible to parasitic attack from bacteria acting normally as saprophytes, such as *Bacillus subtilis, B. liquefaciens, B. mesentericus vulgatus*, and *B. mycoides*, etc. And the excess of oxalic acid induced in the cells by excessive nitrate manuring, when unneutralised by the absorption of calcium, further diminishes its resistance to Root rot. With the restoration of healthy conditions by the aeration of the soil the plants may recover but they never attain their normal growth.

Naturally disease in plants has been studied chiefly, though not exclusively, in our cultivated forms, and in these it is becoming more and more widely spread under the system of intensive culture so much in vogue. The cultivated plants differ widely from the wild forms from which they have been derived, more particularly in the excessive development of parenchymatous tissue and in the reduction of the xylem and sclerenchyma. This modification in cell structure and the large amount of reserves, together with the systems of cultivation which result in rapid growth of succulent tissue, render them specially prone to attack.

In this respect the diseases contracted by plants may be likened to those which have become rife under artificial conditions of modern civilization. In combating these latter much has been done by attending to the laws of hygiene, improved sanitation and rigid enforcement of methods of isolation, to materially lessen the evils due to various bacteria; and *per contra*, in the plant world it is evident that much of our loss is due to neglect of essential precautions and wrong cultural treatment. It cannot be too strongly insisted upon that bacterial diseases of plants are exactly comparable with those of animals, and the same methods which are employed to prevent the spread of anthrax, small-pox, typhoid, or other dangerous zymotic diseases must be followed also in the case of plants. Until this is thoroughly understood and acted upon we shall fail to grapple with the disease problems on our farms and there will be little hope of appreciably diminishing the losses to which the agriculturist is subject.

Just as strict attention should be paid to farm hygiene for plants as for animals. Carefully conducted inoculation experiments, under proper controls, have shown that various beetles, insects, slugs, etc., carry pathogenic germs, and by their bites or by depositing them on wounds

enable these parasites to gain an entrance into the host in a manner parallel to the carriage of malaria by mosquitoes. Many germs are undoubtedly spread by man and the careless handling of diseased material is again responsible for much trouble.

It has been amply proved that susceptibility to disease is influenced by manurial treatment and that high fertilization, especially with nitrogenous manures, lowers the power of the plant to resist infection. Yet the ordinary field experiment continues to be made without reference to this danger. The attempts to estimate the value of the crop by simply noting its yield after the application of a certain amount of manure upon a given area of land are often very misleading and should give way to more scientific methods. The idea that botanical science might be neglected in agriculture and horticulture is now a thing of the past, and we must look for the practical application of the knowledge already gained through the many and important researches in plant physiology and pathology. Much, however, remains to be accomplished; we are not in possession of the cultural and biological facts connected with a great number of the pathogenic bacteria nor, in many cases, do we know their action upon the plant, and until these gaps in our knowledge are filled up many problems must await solution.

In other countries, especially America and Germany, there is enormous activity in this field of research, and the good work done in the numerous plant pathological stations is there recognised as of great economic importance and is applied to great advantage by cultivators in their own interest.

The investigations of botanical workers in the domain of phyto-pathology are also of immense value in their relation to the medical aspects of disease, and I feel convinced that the knowledge now being gained as to the biology of plant tumours and other morbid growths, will be of great service to the investigator in human physiology and pathology and may help in determining the origin and nature of some of the obscure malignant diseases of animal tissues which have proved so baffling a problem in medical science.

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# ON THE CAUSES OF THE HIGH NUTRITIVE VALUE AND FERTILITY OF THE FATTING PASTURES OF ROMNEY MARSH AND OTHER MARSHES IN THE S.E. OF ENGLAND.

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THE coast of Kent and Sussex is to a great extent bordered by flat areas of alluvial land, elevated but little if at all above high water mark, known as the Marshes. Though certain portions are under the plough this land as a rule lies in permanent grass and affords some of the best grazing known in the south of England. The largest of these areas is Romney Marsh which with the adjoining Walland and Denge marshes forms a single area extending about 20 miles from east to west, and 10 miles or so broad. All the marshes possess very similar characteristics and the description which follows of Romney Marsh may be taken to apply, with certain local differences, to the others. The whole of the land has been reclaimed from the sea comparatively recently.

Romney Marsh proper is of pre-Roman origin, but the adjoining marshes which belong to the same sweep have all been " inned " within historical times. Though called a marsh the surface is generally dry and the water level in the ditches by which the land is traversed is three or four feet below the surface. In most of the areas the drainage system is artificial and has to be maintained by opening the sluice gates at low tide. In the main Romney Marsh is occupied by sheep during the summer months, but only the ewes are wintered on the low land, the lambs being taken to the uplands and brought back in spring to be fattened out. Cattle are less seen, though a certain number of bullocks are brought to eat off the rough grass before the grazing season has started. On some of the other marshes cattle are more extensively grazed, indeed the Pevensey level is occupied almost exclusively by cattle. Most of the marshes occur at the mouths of existing rivers and their soils appear to have been formed out of the mud and silt brought down. During the process the course of the river has often changed, for example, the river Rother which now flows out at the western

extremity of the Romney Marsh not many years ago ran practically through the middle of it. The Graveney and other marshes border the estuary of the Medway, and the Richborough and Chislet marshes belong to the Kentish Stour, while each of the Sussex rivers shows a similar alluvial area as it nears the sea. Only the Pevensey level and the alluvial area of West Sussex south of Chichester possess no rivers capable of bringing about the existing deposits of alluvium.

Some indications of the processes by which the marshes were formed may be obtained by observation in the way the land is still growing at the present mouth of the Rother. A bank of shingle and sand is thrown up in the bay and cuts off a lagoon which fills as the tide rises. The still water in this lagoon deposits some of its burden of silt with each tide and the water gradually shallows until a salt marsh is formed that is only flooded at high water. Meantime the shingle bank seawards accumulates sand and becomes a dune, which eventually cuts off the access of the sea, though for many years the lagoon may still be flooded at spring tides. After a considerably longer interval the ridge of shingle and sand, and the flat area behind, in which fine silt has been deposited, both become covered by grass. This however does not represent the whole history of the marsh as may be seen from the fact that almost everywhere throughout Romney Marsh proper there occurs a few feet below present ordinance datum a bed of peat in which are embedded the stumps of a number of trees rooting in a thin bed of blue clay below. Alluvial deposits however exist to a considerable depth below this old forest layer. At the present time we can see in the area no parallel to the conditions which led to this forest growth.

Though the grassland of all the marshes is generally rich it is by no means equally fertile everywhere. In most places one finds fields possessing great local reputation and known to be capable of fattening through the summer months from 6 to 8 or more sheep to the acre without any extraneous assistance. Adjoining these fatting fields there generally exist other fields apparently of the same character but which will do no more than keep sheep in a growing condition, and these differences are well known to the graziers and have been recognised for over a century[1]. In the course of our work on the soils in the south-east of England samples were obtained from Romney Marsh representing the rich and poor pastures respectively, but as the analyses chemical and mechanical fail to reveal any corresponding difference between the soils it was decided to attempt a more thorough investigation of the factors at work.

[1] See for example Price, *Sheep Grazing and Management in Romney Marsh*, 1809.

Accordingly with the assistance of a grant of £150 from the Board of Agriculture a further series of determinations was begun in 1909 and an assistant was stationed in the Marsh during the spring months in order to make the continuous observations that seemed desirable. In three localities fields were found which would afford the desired comparisons, *i.e.* where the fatting field very closely adjoined the poor field, so that both were under very similar conditions of situation and possibly of soil. One of these fields was at Westbroke near Lydd on the land of Mr A. Finn, another was in the east of the Marsh at Orgarswick on the land of Mr H. W. Clements, while the third at Midley on the land of Messrs Davis was also in the west of the Marsh. Samples were also obtained from similar pairs of fields, good and bad, on land at Hope All Saints in Romney Marsh, on land occupied by Mr H. Spanton at Richboro in the Stour Marshes, at Sandhurst in the Rother valley, and again at Oving near Chichester. Only at the Romney Marsh stations however were continuous observations made, and at each of these three centres the work was designed on the same lines. A small square of pasture was railed off and a portion of this was from time to time cut close to represent the grass removed in grazing, the samples being weighed from a given area and sent away for analysis, both botanical and chemical. Soil samples were taken to the depth at which standing water was reached and of these chemical and mechanical analyses were made. Frequent samples were also taken by boring and these were forwarded to the laboratory for determination of their water content. A small bore hole was put down on each plot and the level of the water table was determined each day, an easy matter, for the permanent water was never more than 4 feet from the surface. Regular observations were also made of the water level in the ditches adjoining each field. The temperatures were recorded at a depth of 12 and 6 inches below the surface, in one case by means of automatic recording thermometers, in the others by daily observations with mercurial thermometers. The season of 1909 was cold and the grass was very late to start, but in order to check the results the observations were repeated in 1910 when the season was good throughout May, though it afterwards became abnormally cold and sunless.

It should be premised that the regular custom in grazing Romney Marsh is to begin by stocking the land very heavily. It is considered essential to feed the grass down closely at the outset of a season, and never to allow it to become long or coarse. After the land has thus been " tegged " when the lambs first come down on to the Marsh and

everything has been eaten off close, the more forward lambs are drafted on to the fatting fields at the rate of about 6 to the acre, more being brought in if the season causes an abundance of grass. As the sheep get fat and are sold away, others are drafted in to take their places. Thus, speaking generally, the sheep are always more forward and in fact finishing on the best land, while they are only growing on the poor land. Rarely is any artificial food given, but before lambing time some hay or beans is fed to the ewes on the poor land by the best graziers but not by all. In general whenever either cake or hay is supplied it is to the sheep on the best land.

## Soils.

A very cursory examination of the Marsh shows that it possesses two types of land, a light sand and a heavy loam, which however do not affect the particular problem before us. Throughout the Marsh the old
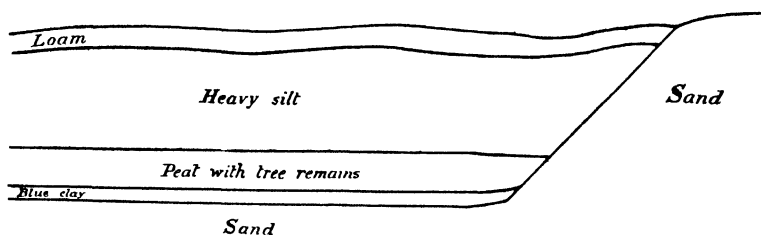


FIG. 1. Section showing formation of Romney Marsh near Lydd.

shingle and sand ridges, the formation of which has been previously indicated, are readily distinguishable. They carry grass which is different in every respect from that of the general meadows, poor in character and readily drying out, nor is it easy to see how it can be improved. The fatting and non-fatting fields to which our enquiries were directed are both situated on the flat silty land and appear to have been formed in exactly the same way. Four layers of material are recognisable; at the surface a loam or clay which forms soil and subsoil proper, below which comes a layer of peat of varying thickness, sometimes containing the tree stumps to which allusion has been made and resting on blue clay, and below that again sand. The depth at which these layers occur differs in the various parts of the Marsh; the accompanying section shows the state of things near Lydd though not upon the land under investigation. At Orgarswick the peat was reached about 7 ft. below the surface but then water came into the bore hole

and no deeper samples could be obtained. At Hope All Saints the upper clay layer was proved to a depth of 7 ft. when the access of water prevented any deeper observations. At Midley the peat was not observed, the clay giving place to sand in the 4th foot from the surface. At Westbroke (Lydd) peat was also not proved and the conditions there were somewhat different from those prevailing in the rest of the Marsh. In the best field the surface was of a much lighter character, only a light sandy loam, and it gave place to pure sand at a depth of 3 ft. In a field of an intermediate character there was about 6 ft. of the same light loam, and then a belt of shingle was entered, beyond which our borings could not penetrate. In the poorest field the sand came right up to the surface. Throughout the greater part of the Marsh the clay from which the soil arises is of a rather heavy type, and gives indications that it has been derived from the Lower Wealden formations over which the Rother runs. It contains about 45 per cent. of fine sand and silt, sometimes the one and sometimes the other predominating, from 15 to 25 per cent. of fine silt, and 25 per cent. of clay particles. It is also rich in carbonate of lime, containing from 4 up to as much as 15 per cent. It should be noted that the Wealden soils *in situ* are practically destitute of carbonate of lime. The sand mainly consists (up to 75 per cent.) of the fine sand fraction with particles of less than 0·2 mm. in diameter, but at Lydd coarse sand was also present to the extent of 10 to 15 per cent. Speaking generally we may say that in the Marsh the surface soil is of a heavy type. Only in a few places, as at Westbroke, does the light type of soil occur, if we leave out of account the banks which represent old lines of dune.

It will now be most convenient to discuss the various points investigated in detail, beginning with the herbage, for though the soil is the main factor determining the herbage, any differences apparent in the latter may suggest the points to which attention should be given in the examination of the soil.

## *Botanical Composition of the Herbage.*

Table 1 shows the composition of the herbage taken from the three stations at Orgarswick, Midley and Westbroke at different dates in 1908, 1909 and 1910, the analyses being made under the supervision of Dr Winifred Brenchley. In all cases the sample taken earlier in the year had been allowed to run up to the flowering stage, and the second sample in the same year was taken from the same spot. The

TABLE 1. *Botanical Analyses of Herbage taken from Romney Marsh Pastures.*

**Orgarswick**

| | June, 1908 | | June 15, 1909 | | July 22, 1909 | | June 22, 1910 | | Sept. 14, 1910 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
| Lolium perenne | 50·6 | 56·6 | 63·68 | 64·03 | 80·45 | 78·38 | 40·07 | 38·81 | 70·04 | 71·52 |
| Agrostis vulgaris and alba | 20·2 | 2·6 | 2·06 | 2·39 | 1·23 | 2·47 | 17·50 | 9·61 | 16·61 | 9·10 |
| Cynosurus cristatus | 15·4 | 15·1 | 14·05 | 18·01 | 9·00 | 9·53 | 9·47 | 13·95 | 0·11 | 0·60 |
| Avena flavescens | 4·3 | 11·8 | 3·63 | 5·98 | 2·60 | 4·15 | 5·84 | 7·81 | 1·78 | 3·32 |
| Hordeum pratense | 0·2 | 4·6 | 1·63 | 4·73 | 1·73 | 2·78 | 0·72 | 2·03 | 0·06 | 0·31 |
| Phleum pratense | 0·4 | 2·0 | 0·27 | 0·26 | 0·30 | 0·27 | — | — | — | — |
| Poa trivialis | 2·1 | 1·2 | 1·86 | 1·84 | 1·40 | 1·09 | 13·62 | 11·30 | 5·68 | 7·02 |
| Holcus lanatus | 0·9 | — | — | 0·07 | — | — | — | 1·16 | 1·56 | 2·34 |
| Dactylis glomerata | — | 0·1 | 2·52 | 0·39 | 1·09 | 0·31 | 0·68 | — | 1·54 | 0·31 |
| Festuca ovina | 0·1 | 0·3 | 0·15 | — | 0·10 | — | — | — | — | — |
| Anthoxanthum odoratum | — | — | — | — | — | — | — | — | — | — |
| Alopecurus agrestis | — | — | — | — | — | — | — | — | 1·01 | — |
| Leguminosae | 2·1 | 0·9 | 1·03 | 0·26 | 1·59 | 0·51 | 6·17 | 8·12 | 1·45 | 3·12 |
| Miscellaneous | 3·7* | 4·8* | 9·07 | 2·02 | 0·46 | 0·47 | 5·90 | 7·17 | 0·08 | 2·31 |

**Westbroke**

| | July 14, 1909 | | June 22, 1910 | | Sept. 14, 1910 | |
|---|---|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
| Lolium perenne | 50·48 | 47·96 | 32·47 | 37·58 | 48·83 | 73·15 |
| Agrostis vulgaris and alba | 13·51 | 21·88 | 23·26 | 11·78 | 11·76 | 11·86 |
| Cynosurus cristatus | 15·75 | 12·02 | 10·82 | 6·12 | 0·34 | 0·37 |
| Avena flavescens | 0·85 | 4·49 | 1·36 | 2·92 | 0·24 | 1·16 |
| Hordeum pratense | — | — | — | 0·75 | — | — |
| Phleum pratense | — | — | — | — | — | — |
| Poa trivialis | 1·35 | 0·28 | 10·68 | 13·41 | 6·43 | 4·74 |
| Holcus lanatus | 10·65 | 0·56 | 12·45 | 0·88 | 28·84 | 0·78 |
| Dactylis glomerata | — | 0·38 | — | 1·36 | — | 1·68 |
| Festuca ovina | — | — | — | — | — | — |
| Anthoxanthum odoratum | — | — | — | — | — | — |
| Alopecurus agrestis | — | — | — | — | — | — |
| Leguminosae | 4·44 | 6·31 | 7·10 | 11·03 | 2·99 | 4·74 |
| Miscellaneous | 2·96 | 6·09 | 1·84 | 14·16 | 0·54 | 1·48 |

**Midley**

| | July 5, 1909 | | June 22, 1910 | |
|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting |
| Lolium perenne | 55·86 | 58·26 | 39·34 | 30·66 |
| Agrostis vulgaris and alba | 4·22 | 3·11 | 8·73 | 12·46 |
| Cynosurus cristatus | 13·41 | 14·32 | 9·06 | 11·17 |
| Avena flavescens | 8·48 | 5·65 | 5·54 | 9·46 |
| Hordeum pratense | 1·69 | 0·77 | — | 2·74 |
| Phleum pratense | 0·85 | 0·35 | — | — |
| Poa trivialis | 6·01 | 4·71 | 13·62 | 7·91 |
| Holcus lanatus | 0·91 | — | 10·10 | 1·34 |
| Dactylis glomerata | — | — | — | 0·41 |
| Festuca ovina | — | — | — | — |
| Anthoxanthum odoratum | — | — | — | — |
| Alopecurus agrestis | — | — | — | — |
| Leguminosae | 7·65 | 1·44 | 12·09 | 8·53 |
| Miscellaneous | 0·87 | 11·37 | 1·49 | 15·31 |

\* Mainly buttercup.

TABLE 1 (continued).

| | Lydd (poor field) | | |
| --- | --- | --- | --- |
| | July 14, 1909 | June 22, 1910 | Sept. 14, 1910 |
| Lolium perenne ............ | 17·76 | 18·79 | 31·55 |
| Agrostis alba and vulgaris | 11·71 | 15·53 | 17·61 |
| Cynosurus cristatus.. ...... | 19·76 | 9·81 | 0·18 |
| Avena flavescens ............ | 4·85 | 3·65 | 2·13 |
| Hordeum pratense ........ | — | 0·26 | — |
| Phleum pratense ............ | — | — | — |
| Poa trivialis ................. | — | 6·52 | 4·16 |
| Holcus lanatus............... | 6·26 | 9·45 | 22·69 |
| Dactylis glomerata ........ | — | — | — |
| Festuca ovina .............. | 5·75 | 0·48 | 0·74 |
| Anthoxanthum odoratum | 4·08 | 9·49 | 7·79 |
| Alopecurus agrestis......... | — | — | — |
| Leguminosae................. | 8·77 | 6·09 | 2·13 |
| Miscellaneous ............. | 16·65 | 18·20 | 10·61 |
| Juncus and Sedges......... | 4·38 | — | — |
| Luzula ...................... | — | 1·69 | 0·38 |

samples taken in different years from the same station were from different spots in the field, so that each had received normal grazing treatment in the previous years. It will be noticed that the prominent grass in all cases is *Lolium perenne*, which constitutes from one-third up to four-fifths of the total herbage; *Agrostis* is also a regular constituent of the herbage and rises on occasion to over 20 per cent. Crested Dogstail is also abundant in the first cuttings, but almost disappears in the later growth. *Poa trivialis* was also abundant in 1910 though little of it was to be seen in the preceding year. This grass also bulks more in the earlier than in the later cuts. *Holcus lanatus* is abundant in the Westbroke herbage on the fatting fields and also on one occasion in the non-fatting field at Midley. The Leguminosae never became very prominent in the herbage analysed, but all the fields contain a good deal of white clover though little gets into the samples taken away for analysis, because of its low creeping habit. The pastures are not particularly weedy though on occasion the miscellaneous species chiefly buttercups rise to 15 per cent. One fact brought out by these analyses is the way the floral type of the same field varies from year to year. For example (considering the first cut only) the *Agrostis* at Orgarswick was 20 per cent. in 1908, 2 per cent. in 1909 and 17 per cent. in 1910, and *Holcus* constituted only 1 per cent. of the Midley herbage in 1909 and 11 per cent. in 1910. Comparing the fatting and non-fatting fields it

is remarkable how similar the floral type is. Each pair shows an almost exactly similar analysis on every occasion. In particular the *Lolium perenne* on the non-fatting and fatting fields agrees within the limits of error of the determination, in every case except one, and the Crested Dogstail behaves in a similar manner. With one exception *Avena flavescens* is always more abundant on the non-fatting fields, as also is *Hordeum pratense*. Whenever *Holcus lanatus* occurs it is more abundant on the fatting fields, the excess being very marked at Midley and Westbroke. The distribution of the Leguminosae is quite irregular, sometimes the fatting and sometimes the non-fatting field yields the greater proportion. The distribution of the weeds is also irregular, but at Midley and Westbroke whenever they bulk largely in the herbage they are more abundant on the non-fatting fields.

As regards the floral type we may say at once that there are no differences which would account for the superior feeding value of the herbage from the fatting field. Such differences as exist are rarely significant and are trivial in comparison with the great general agreement between the pairs of fields. It is clear then that the value of the pasture is not determined by the floral type of the herbage. Botanical analysis, however, gives a very imperfect picture of the differences to be noted in the pairs of fields. On the spot it is at once observable that the surface of the good land is much better covered, there is no bare soil to be seen, and the turf possesses that indefinable springiness characteristic of the best grass land. But the most notable difference is that on the good land the grasses are essentially leafy, with broad blades and much less tendency to run up to flowering heads. On the non-fatting fields the herbage is markedly stemmy, the leaves are fewer and narrower, and the flower heads come early and abundantly. For example, in May the poor fields are generally conspicuously covered with buttercups, but on analysis the buttercup herbage is equally abundant on the good land though it does not betray itself to the eye by throwing up flowers. This leafiness and stemminess respectively constitutes the chief difference between the herbage of the two classes of field, and it is independent of the floral type, which, as we have said before, varies but little in passing from the good to the poor field. Two other differences can be observed: the fatting field obviously contains more clover than the non-fatting field, and during summer its whole herbage remains green whilst that on the non-fatting field tends to become brown and burned so that the Bent grass shows up conspicuously.

Table 2 gives a similar comparison between a good and a poor field from one of the Rother levels leading into Romney Marsh. These, however, are not sheep pastures, but are fed off with cows. The good one is reported by the occupier to enable the cows to yield milk freely even when fed right down to the grass roots, whereas the poor field yields little milk though it will keep young stock in fair condition. The difference in management is reflected at once in a changed floral type; *Lolium perenne* is no longer a dominant grass as on the sheep

TABLE 2. *Botanical analyses of herbage from adjacent good and poor cow pasture. Rother Level.*

| | 1909. Rother Level, fringe of Romney Marsh | |
| --- | --- | --- |
| | Good | Poor |
| Lolium perenne ............... | 6·82 | 2·10 |
| Agrostis vulgaris ............ | 36·69 | 29·71 |
| Cynosurus cristatus ......... | 12·84 | 11·63 |
| Avena flavescens............... | 0·54 | 8·05 |
| Hordeum pratense............ | 0·26 | 0·78 |
| Poa trivialis..................... | 0·58 | — |
| Holcus lanatus ............... | 11·89 | 16·39 |
| Dactylis glomerata............ | 0·56 | — |
| Festuca ovina.................. | 0·18 | 1·41 |
| Anthoxanthum odoratum... | 5·63 | 5·10 |
| Alopecurus agrestis ......... | 1·76 | — |
| Leguminosae .................. | 8·62 | 19·19 |
| Miscellaneous .................. | 13·58 | 5·61 |

pastures, *Agrostis*, Crested Dogstail and *Holcus* being most abundant. But again the floral type on the good and poor fields is almost identical, the only pronounced differences being a greater proportion of Leguminosae and a smaller proportion of weeds on the poor field and not on the good field as might *a priori* have been expected. The habit of growth shows the same differences as before, the good herbage being leafy and the poor herbage stemmy.

*Properties of the Soils.*

*Orgarswick.* Table 3 shows the mechanical analyses of the soil from the fatting and non-fatting fields respectively at Orgarswick for each successive foot down to 7 ft. from the surface. An inspection of these figures shows that the soil is exceptionally uniform in composition, the seventh differing but little from the top. The type is a heavy one

TABLE 3. *Mechanical Analyses of Soils, from Fatting and Non-fatting Fields.*

### Orgarswick Fatting Field.

|  | 1st 12 ins. | 2nd 2 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. | 7th 12 ins. |
|---|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | nil | 0·1 | 0·1 | 0·2 | 0·1 | 0·1 |
| Coarse sand, 1—0·2 mm. | 0·2 | 0·2 | 0·3 | 0·6 | 0·3 | 0·2 | 0·8 |
| Fine sand, 0·2—0·04 mm. | 27·6 | 27·0 | 23·5 | 29·3 | 20·3 | 16·7 | 25·9 |
| Silt, 0·04—0·01 mm. ...... | 18·0 | 18·9 | 19·0 | 17·0 | 18·5 | 17·1 | 17·8 |
| Fine silt, 0·01—0·002 mm. | 16·0 | 14·9 | 14·9 | 13·4 | 15·7 | 15·9 | 15·4 |
| Clay, below 0·002 mm. ... | 21·8 | 25·0 | 25·6 | 22·6 | 26·6 | 30·5 | 23·9 |
| Calcium carbonate ......... | nil | 2·5 | 6·9 | 9·5 | 7·8 | 9·6 | 6·4 |
| Loss on ignition ............ | 8·7 | 7·0 | 5·8 | 5·4 | 6·1 | 5·7 | 7·0 |
| Moisture ..................... | 3·9 | 3·6 | 2·6 | 2·1 | 2·3 | 3·4 | 2·6 |

No stones

### Orgarswick Non-fatting Field.

|  | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. | 7th 12 ins. |
|---|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | 0·1 | 0·1 | 0·1 | 0·1 | 0·0 | 0·1 |
| Coarse sand, 1—0·2 mm. | 0·1 | 0·6 | 0·1 | 0·1 | 0·2 | 0·1 | 0·2 |
| Fine sand, 0·2—0·04 mm. | 25·4 | 26·7 | 30·7 | 23·6 | 18·0 | 25·5 | 22·1 |
| Silt, 0·04—0·01 mm. ...... | 18·1 | 19·0 | 18·6 | 18·1 | 17·7 | 15·5 | 19·4 |
| Fine silt, 0·01—0·002 mm. | 16·6 | 15·1 | 12·4 | 14·9 | 16·8 | 14·4 | 15·6 |
| Clay, below 0·002 mm. ... | 24·0 | 25·8 | 20·9 | 22·6 | 24·2 | 25·2 | 25·4 |
| Calcium carbonate ......... | 0·3 | 1·4 | 7·6 | 11·0 | 8·2 | 7·9 | 7·9 |
| Loss on ignition ............ | 7·7 | 6·2 | 8·1 | 9·5 | 5·6 | 6·2 | 5·5 |
| Moisture ..................... | 4·1 | 3·6 | 2·1 | 2·1 | 2·6 | 2·6 | 2·2 |

No stones

### Westbroke Fatting Field.

|  | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | 0·1 | 0·3 | nil | nil | nil |
| Coarse sand, 1—0·2 mm. | 2·3 | 1·1 | 0·5 | 5·7 | 13·6 | 17·7 |
| Fine sand, 0·2—0·04 mm. | 57·6 | 58·4 | 59·8 | 71·9 | 70·2 | 66·3 |
| Silt, 0·04—0·01 mm. ...... | 11·9 | 11·6 | 11·1 | 5·6 | 3·1 | 3·2 |
| Fine silt, 0·01—0·002 mm. | 8·6 | 7·3 | 5·4 | 2·1 | 2·5 | 2·2 |
| Clay, below 0·002 mm. ... | 8·0 | 9·9 | 8·5 | 4·2 | 3·0 | 3·1 |
| Calcium carbonate ......... | 0·2 | 4·4 | 9·6 | 7·5 | 4·5 | 8·4 |
| Loss on ignition ............ | 6·4 | 3·0 | 3·1 | 2·2 | 2·0 | 0·2 |
| Moisture ..................... | 2·7 | 2·1 | 1·3 | 1·2 | 1·9 | 1·9 |

Few stones in surface soil only

TABLE 3 *(continued).*

## Westbroke Non-fatting Field.

| | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. |
|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·2 | 0·1 | 0·0 | 0·9 |
| Coarse sand, 1—0·2 mm. | 2·0 | 0·9 | 0·8 | 5·2 |
| Fine sand, 0·2—0·04 mm. | 35·4 | 28·9 | 18·9 | 18·1 |
| Silt, 0·04—0·01 mm. ...... | 22·4 | 18·2 | 18·9 | 18·3 |
| Fine silt, 0·01—0·002 mm. | 9·9 | 14·5 | 14·4 | 14·7 |
| Clay, below 0·002 mm. ... | 16·3 | 24·8 | 27·7 | 30·1 |
| Calcium carbonate ......... | 0·1 | 1·1 | 3·7 | 2·0 |
| Loss on ignition ............ | 8·1 | 5·4 | 6·7 | 9·8 |
| Moisture ..................... | 3·5 | 3·5 | 2·8 | 1·7 |

Few stones in surface soil only

## Lydd Poor Field.

| | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | 0·2 | 0·1 | 0·1 | 0·2 | 0·0 |
| Coarse sand, 1—0·2 mm. | 3·1 | 1·7 | 1·0 | 3·4 | 8·2 | 9·7 |
| Fine sand, 0·2—0·04 mm. | 67·5 | 71·6 | 73·4 | 75·3 | 73·1 | 73·3 |
| Silt, 0·04—0·01 mm. ... .. | 6·2 | 6·8 | 4·8 | 3·3 | 2·5 | 2·3 |
| Fine silt, 0·01—0·002 mm. | 6·9 | 5·5 | 3·9 | 3·3 | 3·1 | 2·5 |
| Clay, below 0·002 mm. ... | 5·1 | 6·1 | 4·7 | 4·4 | 3·6 | 3·8 |
| Calcium carbonate ...... .. | 0·2 | 1·4 | 5·2 | 8·7 | 5·2 | 5·2 |
| Loss on ignition ............ | 9·5 | 3·8 | 2·9 | 3·7 | 3·4 | 3·1 |
| Moisture ..................... | 2·3 | 1·6 | 2·3 | 1·9 | 1·8 | 1·7 |

Few stones

## Midley Fatting Field.

| | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | 0·1 | 0·1 | 0·0 | 0·2 | No sample taken |
| Coarse sand, 1—0·2 mm. | 0·5 | 0·3 | 0·3 | 0·4 | 0·7 | |
| Fine sand, 0·2—0·04 mm. | 47·8 | 36·1 | 47·9 | 69·2 | 73·9 | |
| Silt, 0·04—0·01 mm. | 12·8 | 14·5 | 11·1 | 6·3 | 4·9 | |
| Fine silt, 0·01—0·002 mm. | 11·9 | 14·1 | 10·3 | 6·4 | 8·7 | |
| Clay, below 0·002 mm. ... | 13·9 | 19·0 | 13·8 | 6·8 | 4·9 | |
| Calcium carbonate ......... | 0·1 | 3·6 | 4·4 | 6·3 | 7·0 | |
| Loss on ignition ............ | 6·6 | 7·5 | 7·3 | 4·3 | 3·0 | |
| Moisture ..................... | 3·7 | 2·9 | 1·7 | 0·8 | 0·8 | |

No stones

TABLE 3 *(continued)*.

## Midley Non-fatting Field.

|  | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·2 | 0·0 | 0·1 | 0·1 | 0·0 | 0·0 |
| Coarse sand, 1—0·2 mm. | 0·5 | 0·4 | 0·2 | 0·2 | 0·5 | 0·6 |
| Fine sand, 0·2—0·04 mm. | 38·0 | 38·3 | 56·7 | 66·9 | 74·2 | 75·2 |
| Silt, 0·04—0·01 mm. ...... | 13·8 | 15·0 | 9·6 | 7·7 | 5·6 | 4·5 |
| Fine silt, 0·01—0·002 mm. | 13·5 | 12·6 | 7·9 | 6·9 | 5·4 | 4·0 |
| Clay, below 0·002 mm. ... | 19·1 | 19·1 | 11·4 | 6·9 | 4·7 | 4·3 |
| Calcium carbonate ......... | 0 3 | 4·2 | 6·2 | 7·0 | 7·2 | 6·6 |
| Loss on ignition ............ | 9·1 | 6·3 | 6·9 | 4·0 | 3·2 | 0 8 |
| Moisture ..................... | 3·0 | 3·4 | 1·2 | 1·6 | 1·7 | 1·5 |

No stones

## Hope All Saints Fatting Field.

|  | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | 0·1 | 0·1 | 0·1 | 0·1 | nil | nil |
| Coarse sand, 1—0·2 mm. | 1·3 | 0·8 | 0·2 | 0·7 | 0·4 | 0·2 |
| Fine sand, 0·2—0·04 mm. | 31·1 | 27·1 | 18·0 | 22·4 | 23·4 | 25·0 |
| Silt, 0·04—0·01 mm. ...... | 18·2 | 17·1 | 19·0 | 16·3 | 15·7 | 17·3 |
| Fine silt, 0·01—0·002 mm. | 11·0 | 13·4 | 18·9 | 11·2 | 10·3 | 9·7 |
| Clay, below 0·002 mm. ... | 19·5 | 17·9 | 19·8 | 23·7 | 22·1 | 21·0 |
| Calcium carbonate ......... | 0·7 | 8·0 | 13·1 | 11·5 | 12·7 | 13·4 |
| Loss on ignition ............ | 9·5 | 9·4 | 12·2 | 9·53 | 9·0 | 8·7 |
| Moisture ..................... | 4·7 | 3·4 | 2·8 | 1·8 | 1·6 | 2·8 |

No stones

## Hope All Saints Non-fatting Field.

|  | 1st 12 ins. | 2nd 12 ins. | 3rd 12 ins. | 4th 12 ins. | 5th 12 ins. | 6th 12 ins. |
|---|---|---|---|---|---|---|
| Fine gravel, above 1 mm. | nil | nil | nil | nil | nil | nil |
| Coarse sand, 1—0·2 mm. | 0·2 | 0·4 | nil | 0·1 | 0·1 | 0·2 |
| Fine sand, 0·2—0·04 mm. | 18·0 | 14·2 | 9·3 | 20·4 | 20·7 | 26·3 |
| Silt, 0·04—0·01 mm. ...... | 19·8 | 19·3 | 21·7 | 15·9 | 22·2 | 20·4 |
| Fine silt, 0·01—0·002 mm. | 19 5 | 19·4 | 18·6 | 8·9 | 10·9 | 11·1 |
| Clay, below 0·002 mm. ... | 26·2 | 32·9 | 33·1 | 25·7 | 24·4 | 23·0 |
| Calcium carbonate ......... | 0·2 | 0·1 | 3·7 | 10·8 | 14·5 | 10·8 |
| Loss on ignition ..... ...... | 9 3 | 5·3 | 7·4 | 8·6 | 7·1 | 7·8 |
| Moisture ..................... | 4·8 | 5·0 | 2·9 | 2·5 | 2·4 | 2·2 |

characterised by an entire absence of stones and by only a minute fraction of coarse sand. The clay constitutes about one quarter of the whole soil and the fine silt is also an exceptionally high fraction, averaging about 15 per cent. Such proportions are also characteristic of the Lower Wealden soils in the basin of the Rother. A comparison of the fatting and non-fatting fields shows no significant differences, the results given by the two soils agreeing within the limits of error of the method. It should be noted, however, as the analyses are made upon samples each representing the average soil in a layer a foot thick, the presence of a thin parting of pure clay would be overlooked since it would not appreciably raise the percentage of clay in the whole layer, though it might seriously interfere with the movement of the water upwards or downwards. Below the seventh foot peat was reached which was saturated with water so that the boring could not be continued. Table 4 shows a determination of the water content of the soil at various dates during 1909–10. Again there is no marked difference between the fatting and non-fatting field though there is evidence that the soil of the fatting field is rather moister than the other in the early summer and becomes somewhat drier as the season advances, as though the extra growth of grass had caused an increased transpiration of

TABLE 4. *Moisture content of Orgarswick soils. Percentages.*

|  | May 31, 1909 | | July 2, 1909 | | July 20, 1909 | |
|  | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
|---|---|---|---|---|---|---|
| 1st foot | 13·2 | 15·7 | 16·7 | 17·3 | 16·9 | 20·3 |
| 2nd ,, | 14·1 | 15·5 | 11·0 | 14·0 | 14·5 | 12·6 |
| 3rd ,, | *18·4 | †19·3 | 16·8 | 16·5 | 16·8 | 16·6 |

|  | March 16, 1910 | | May 13, 1910 | | June 22, 1910 | | Sept. 14, 1910 | |
|  | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
|---|---|---|---|---|---|---|---|---|
| 1st foot | 29·3 | 26·8 | 30·9 | 28·4 | 23·0 | 20·8 | 15·3 | 23·3 |
| 2nd ,, | 22·9 | 21·2 | 22·9 | 23·0 | 18·7 | 15·6 | 13·3 | 19·1 |
| 3rd ,, | 22·8 | 20·2 | 22·7 | 22·4 | 18·9 | 24·1 | 15·1 | 20·2 |
| 4th ,, | Water level | 20·2 | 23·2 | 22·4 | 21·6 | 21·1 | 23·1 | 21·4 |
| 5th ,, |  | 22·1 |  |  |  |  |  |  |

* Water level was reached here.   † Water level was 6 ins. lower down.

TABLE 4 (*continued*). *Midley soils.*

| | June 7, 1909 | | July 23, 1909 | | March 17, 1910 | | May 13, 1910 | | June 22, 1910 | | Sept. 14, 1910 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
| 1st 12 ins. | 18·4 | 18·1 | 22·7 | 18·5 | 27·9 | [31·5] | 27·5 | 28·7 | 22·3 | 17·3 | 16·6 | 19·4 |
| 2nd ,, | 14·8 | 15·6 | 14·2 | 14·8 | 23·8 | 25·3 | 21·9 | 22·6 | 18·2 | 17·4 | 15·4 | 17·5 |
| 3rd ,, | 17·3 | 18·4 | 14·7 | 19·5 | 23·3 | 23·2 | 22·3 | 23·7 | 20·3 | 21·3 | 18·7 | 21·0 |
| 4th ,, | 18·9 | 19·5* | 19·0 | 21·1 | Water | 23·8 | 22·1 | 22·5 | 20·4 | 22·7 | 20·2 | 21·6 |

*Lydd soils.*

| | Westbroke, June 8, 1909 | | Lydd | Westbroke, July 14, 1909 | | Lydd poor | Westbroke, July 26, 1909 | | Lydd |
|---|---|---|---|---|---|---|---|---|---|
| | Fatting | Non-fatting | Non-fatting | Fatting | Non-fatting | | Fatting | Non-fatting | Non-fatting |
| 1st 12 ins. | 18·4 | | 13·3 | 21·8 | | 21·4 | 16·6 | 19·1 | 8·1 |
| 2nd ,, | 14·4 | | 16·5 | 13·7 | | 20·1 | 15·2 | 15·3 | 12·4 |
| 3rd ,, | 18·9 | | 16·5 | 19·6 | | 22·1 | 19·2 | 15·6 | 18·5 |
| 4th ,, | 18·9 | | 17·9* | 20·7 | | 22·7 | 20·5 | 17·4 | 21·0 |

\* 6 ins. only.

| | Westbroke, March 16, 1910 | | Lydd poor | Westbroke, May 14, 1910 | | Westbroke, June 22, 1910 | | Westbroke, Sept. 15, 1910 | | Lydd poor |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fatting | Non-fatting | | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | |
| 1st 12 ins. | 27·0 | 25·8 | 24·4 | 29·0 | 29·4 | 26·4 | 13·2 | 15·3 | 16·0 | 10·5 |
| 2nd ,, | 22·2 | 20·2 | 19·1 | 17·5 | 20·5 | 27·1 | 16·2 | 14·7 | 15·7 | 9·5 |
| 3rd ,, | 21·0 | 20·5 | 21·3 | 19·0 | 18·0 | 17·5 | 17·3 | 17·7 | 19·1 | 18·8 |
| 4th ,, | (water) | 16·6 | 21·7 | 23·8 | | 18·1 | 26·4 | 14·1 | 24·2 | 19·6 |
| 5th ,, | | (water at 3′ 6″, stones in sample) | 21·4 | | | | | | | |

water drawn from the soil. Table 5 shows the weekly mean of the daily temperature readings during the growing season of 1909 taken some at the depth of 6 inches and throughout the whole period also at the depth of 12 inches. The differences are small but they all tend to

TABLE 5.  *Orgarswick Soil Temperatures, °C.  Average of daily readings.*

| Date 1909 | At 6 ins. | | At 12 ins. | | Air temperature |
|---|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting | |
| May 20—26......... | 12·0 | | 12·4 | 11·8 | 18·1 |
| ,,  27—June 2... | 13·9 | | 13·0 | 12·8 | 18·6 |
| June 3—9  ........ | 12·8 | | 12·6 | 12·7 | 14·7 |
| ,,  10—16  ..... | 12·9 | | 12·6 | 12·7 | 16·1 |
| ,,  17—23  ..... | 14·8 | | 13·8 | 13·6 | 18·7 |
| ,,  24—30  ..... | 14·4 | 14·1 | 14·1 | 13·9 | 17·0 |
| July 1—7............. | *15·1 | *15·0 | *14·4 | *14·3 | 18·6 |
| ,,  8—14  ........ | *15·4 | *15·4 | *14·9 | *14·6 | 18·4 |
| ,,  15—21......... | *17·1 | *16·9 | *15·4 | *15·2 | 21·7 |

Midley.

| 1909 | Soil temperature 6 ins. | | Air temperature |
|---|---|---|---|
| | Fatting | Non-fatting | |
| (1) June 16—23 | 15·3 | 15·7 | 18·3 |
| ,,  24—30 | 14·3 | 14·7 | 16·0 |
| July  1—7 | 14·6 | 15·4 | 17·7 |
| (2)  ,,  8—14 | 15·4 | 15·2 | 17·3 |
| (2)  ,,  15—21 | 16·6 | 16·4 | 20·1 |

| 1909 | Soil temperature 6 ins. Westbroke | | Lydd poor | Air temperature |
|---|---|---|---|---|
| | Fatting | Non-fatting | | |
| June 10—16 | *15·3 | *16·8 | | 16·0 |
| ,,  17—23 | 16·3 | 17·2 | †17·3 | 19·0 |
| ,,  24—30 | 15·1 | 15·7 | 15·6 | 16·1 |
| July  1—7 | 15·9 | 16·3 | 16·7 | 18·2 |
| .,,  8—14 | 14·8 | 15·6 | 15·4 | 19·6 |
| ,,  15—21 | *16·6 | *16·6 | *16·2 | 20·6 |

6 days only.                    † 5 days only.

show that the soil of the fatting field is slightly warmer than that of the non-fatting field. Recording thermometers were also set in the soil at the depth of 6 inches at these stations. The traces bring out the same general result—that there is a slight constant difference in temperature in favour of the fatting field. One other fact is also apparent, that the amplitude of the diurnal fluctuation of temperature is rather greater on the non-fatting field, though again the differences are not very pronounced. Table 6 shows the chemical composition of the pairs of soils. Again the results are very much alike. The subsoil contains an abundance of calcium carbonate, though in the surface layer this

TABLE 6. *Chemical Analysis of Soils from Orgarswick, percentages.*

| | 12 ins. Samples | | | | 9 ins. Samples | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st Depth | | 2nd Depth | | 1st Depth | | 2nd Depth | |
| | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting | Fatting | Non-fatting |
| Nitrogen | 0·365 | 0·209 | 0·170 | 0·142 | 0·445 | 0·383 | 0·212 | 0·184 |
| $K_2O$ soluble in conc. HCl | 0·541 | 0·514 | 0·549 | 0·515 | 0·56 | 0·66 | 0·77 | 0·92 |
| $K_2O$ soluble in 1 °/₀ citric acid | | | | | 0·044 | 0·070 | | |
| $P_2O_5$ soluble in conc. HCl | 0·133 | 0·098 | 0·116 | 0·095 | 0·149 | 0·116 | 0·127 | 0·083 |
| $P_2O_5$ soluble in 1 °/₀ citric acid | | | | | 0·010 | 0·007 | | |
| $Al_2O_3$ | | | | | 3·89 | 4·91 | | |
| $Fe_2O_3$ | | | | | 3·62 | 3·82 | | |
| $Mn_3O_4$ | 0·029 | 0·035 | 0·062 | 0·024 | 0·08 | 0·18 | | |
| CaO | | | | | 0·80 | 0·86 | | |
| MgO | | | | | 0·64 | 0·71 | | |
| Carbonate (expressed as $CaCO_3$) | nil | 0·32 | 2·52 | 1·36 | 0·10 | 0·42 | 0·33 | 0·60 |

| | Midley soils | | | |
|---|---|---|---|---|
| | Fatting | Non-fatting | Fatting | Non-fatting |
| | 1st 12 ins. | 1st 12 ins. | 2nd 12 ins. | 2nd 12 ins. |
| Nitrogen | 0·358 | 0·352 | 0·164 | 0·153 |
| $K_2O$ soluble in conc. HCl | 0·560 | 0·569 | 0·619 | 0·510 |
| $P_2O_5$   ,,     ,,     ,, | 0·145 | 0·124 | 0·126 | 0·109 |
| $Mn_3O_4$ | 0·054 | 0·079 | 0·051 | 0·062 |
| Carbonate (expressed as $CaCO_3$) | 0·11 | 0·34 | 3·63 | 4·18 |

TABLE 6 (*continued*).

| Westbroke | Fatting | Non-fatting | Fatting | Non-fatting |
|---|---|---|---|---|
| | 1st 12 ins. | 1st 12 ins. | 2nd 12 ins. | 2nd 12 ins. |
| Nitrogen | 0·309 | 0·346 | 0·145 | 0·163 |
| K₂O soluble in conc. HCl | 0·409 | 0·541 | 0·472 | 0·568 |
| P₂O₅  ,,  ,,  ,, | 0·124 | 0·104 | 0·087 | 0·094 |
| Mn₃O₄ | 0·031 | 0·029 | 0·065 | 0·045 |
| Carbonate (expressed as CaCO₃) | 0·23 | 0·13 | 4·44 | 1·08 |

| Westbroke 9 ins. samples | Fatting | Lydd poor |
|---|---|---|
| Moisture | 4·05 | 2·71 |
| Loss on ignition | 9·26 | 7·43 |
| Nitrogen | 0·356 | 0·296 |
| Al₂O₃ | 1·93 | 1·72 |
| Fe₂O₃ | 3·00 | 2·55 |
| Mn₃O₄ | nil | nil |
| MgO | 0·32 | 0·30 |
| CaO | 0·36 | 0·37 |
| Carbonates (expressed as CaCO₃) | nil | 0·13 |
| K₂O soluble in conc. HCl | 0·28 | 0·26 |
| K₂O soluble in 1 °/₀ citric acid | 0·014 | 0·055 |
| P₂O₅ soluble in conc. HCl | 0·126 | 0·101 |
| P₂O₅ soluble in 1 °/₀ citric acid | 0·008 | 0·007 |
| SO₃ | 0·07 | 0·07 |

constituent has been considerably reduced in amount through removal by solution, indeed on the poor soil some sorrel occurs. Chemically the two soils may be regarded as identical, most of the differences being within the limits of error of sampling and analysis. It should however be noticed that there is a little more nitrogen and particularly a little more phosphoric acid in the soil of the good field, though that of the poor field still contains quantities which would be regarded as large were nothing known about the soil. Thus as regards the mechanical and chemical composition, temperature and moisture determinations, little can be found to discriminate between the two soils, and though some of the factors of production are slightly better in the good soil the differences seem too small to be significant.

On subjecting the soils to more refined methods of treatment more pronounced differences were observed. It has already been stated that

the herbage of the fatting field starts into growth at an earlier date, proceeds more rapidly and possesses a darker green colour and wider leaves, sufficiently so to cause a marked difference in the aspect of the two fields. The differences are such as might be expected from a larger supply of nitrate to the herbage on the fatting field, and analysis of the soil demonstrates the fact that in the early part of the season the soil of the fatting field does contain more nitrate and ammonia, though this difference disappears as the season advances. Table 7 gives the figures for samples of soil taken in 1910. It was found also by

TABLE 7.  *Nitrogen as nitrate*[1] *and ammonia, parts per million of dry soil.*  1910.

| | | March 16 | | May 13 | | June 22 | Sept. 14 |
|---|---|---|---|---|---|---|---|
| | | Nitrate | Ammonia | Nitrate | Ammonia | Nitrate | Nitrate |
| **Orgarswick.** | | | | | | | |
| *Surface soil.* | Fatting | 15·8 | 8·2 | 14·6 | 7·2 | 1·4 | 9·4 |
| | Non-fatting | 10·4 | 8·5 | 8·9 | 2·8 | 3·0 | 58·4[2] |
| **Midley.** | | | | | | | |
| *Surface soil.* | Fatting | 12·4 | 7·3 | 24·2 | 6·5 | 6·4 | 15·4 |
| | Non-fatting | 6·3 | 10·9 | 12·5 | 2·8 | 2·7 | 21·7 |
| **Westbroke.** | | | | | | | |
| *Surface soil.* | Fatting | 23·6 | 7·3 | 21·5 | 20·8 | 2·3 | 9·4 |
| | Non-fatting | 13·0 | 6·2 | 7·9 | 6·0 | 2·0 | 8·2 |
| | Lydd poor | 11·0 | 12·1 | 4·1 | 7·8 | 5·2 | 15·7 |

direct experiment that the rate of nitrate production in the soil of the fatting field was greater than that on the non-fatting field. These results are reflected in the yields of grass from the two fields which were measured by cutting over a small fixed area. The determinations could only be very approximate, but they showed conclusively that much more grass grows on the fatting field in response to the increased food supply though the floral type is not altered thereby.

[1] The nitrates are determined by washing the soil with water, boiling the extract with magnesia to expel ammonia, and then reducing with a zinc-copper couple. The results are somewhat too high, as they include small quantities of other soluble nitrogen compounds reducible under these conditions, but for our present purpose they are more useful than values of nitrate alone would be.

[2] This result is wholly abnormal and probably arises through the inadvertent inclusion of spots where sheep had recently staled.

The fact that decomposition of the soil organic matter proceeded more rapidly on the fatting than on the non-fatting field was also proved by analysis of the soil gases. At each centre there was more carbon dioxide in the gas extracted from the fatting field than in the gas from the non-fatting field: here (at Orgarswick) the figures were 1·1 and 0·7 per cent. respectively.

A study of the water level in the borings in the two fields and in the ditch also shows a certain small but constant difference. The fatting field is very slightly higher than the non-fatting field, the difference being almost exactly 6 inches between the two points at which observations were made. A difference of 6 inches in the elevation may seem trifling but the land is only about 10 to 20 ft. above sea level, and the water table is only about 40 inches from the surface.
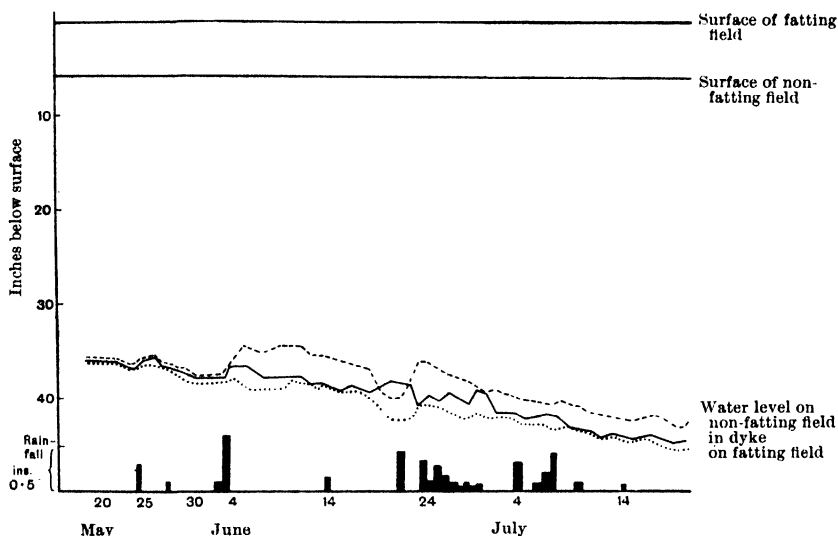


Fig. 2. Rainfall and water level at Orgarswick.

Figure 2 shows the relative position of the surface and of the standing water below through the season of 1909. In May the water level below the two fields and in the ditch between them all stood at the same height, but as the season advanced it fell in all three, most rapidly in the well in the fatting field, almost equally rapidly in the dyke, but much less rapidly in the well in the non-fatting field. At starting the water table was 36 inches from the surface of the fatting field, and 31 inches from the surface of the non-fatting field, towards the end of July the distance of the surface of the fatting field to water was 46

inches, whereas it was only 37 inches in the non-fatting field. Thus the soil tends to be kept a little drier in the fatting field, there being always a greater distance between the surface and standing water. Furthermore, the fluctuations of the curve due to rain would seem to show that water sinking from the surface gets away rather more quickly and thoroughly from the soil of the fatting field. For instance the heavy rains of June 4th and 22nd, which permanently raised the water table below the non-fatting field, did not much affect the level below the fatting field. This would seem to indicate some difference in texture in the soil not brought out by the mechanical analyses, and this was confirmed by the fact that on the non-fatting field the soil felt harder beneath the feet and it was markedly more difficult to bore out the samples. Again, when samples were made down to the water table the bore hole on the fatting field would always fill up with water much more rapidly than that on the non-fatting field. These differences of level, drainage and relative position of the water table would have a much more potent effect upon the herbage in early years before the Marsh was so thoroughly drained as it is at the present time.

### *The Midley soils.*

Here the sand lies much closer to the surface than at Orgarswick and there is a more distinct difference between the top twelve inches on the good and bad fields (Table 3). The surface soil on the good field is distinctly lighter than on the bad field, and might be described rather as a loam than as a clay; below this, however, the soils are similar in both fields. Even then the heavier soil on the poor field is somewhat lighter than, though very similar in type to that of Orgarswick. As at Orgarswick there is an abundance of calcium carbonate in the subsoil but it has been mostly washed away from the surface soil; there are no stones and practically no coarse sand on either sample. No very consistent differences are to be found in the water content (Table 4), even in the top foot where the heavier texture might be expected to cause the retention of more water. The differences in the mean weekly temperatures (Table 5) are unimportant, but the good field shows in the earlier period a slightly lower temperature than the poor field, which is the reverse of what was found at Orgarswick. In chemical composition the two soils resemble one another much more closely than do the two soils at Orgarswick; the only noticeable difference comes in the phosphoric acid, which is again higher in the soil of the better field. As

regards levels it is again found that the fatting field is somewhat higher (8·2 in.) than the non-fatting field and the water table is always somewhat further from the surface.

Table 8 shows the relative depths of the water table with regard to the surface, and the last column gives the absolute difference of the level of the water in the two fields. It will be noticed that the water level in the non-fatting field is actually lower than in the fatting field, while both at Orgarswick and at Lydd it was higher. This difference may be

TABLE 8. *Level of water in wells, average of daily readings. Midley.*
(The non-fatting plot is 8·2 ins. below the fatting plot.)

| | Fatting | Non-fatting | Level of water in fatting well below non-fatting surface | Amount by which water level is lower in non-fatting than in fatting field |
|---|---|---|---|---|
| | ins. | ins. | ins. | ins. |
| May 20th—26th ...... | 50·7 | 43·0 | 42·5 | ·5 |
| ,, 27th—June 2nd | 51·9 | 45·4 | 43·7 | 1·7 |
| June 3rd—9th ......... | 53·2 | 45·4 | 45·0 | ·4 |
| ,, 10th—16th ...... | 55·0 | 46·1 | 46·8 | − ·7 |
| ,, 17th—23rd ...... | 55·2 | 48·3 | 47·0 | 1·3 |
| ,, 24th—30th ...... | 56·0 | 48·7 | 47·8 | ·9 |
| July 1st—7th ......... | 58·4 | 49·6 | 50·2 | − ·6 |
| ,, 8th—14th........ | * 58·7 | 50·8 | 50·5 | ·3 |
| ,, 15th—21st ...... | (well dry) | 51·8 | | |

* 4 days only.

attributed to the position of the dyke: at this station the dyke did not separate the two fields but ran only on the one side of the non-fatting field. The water table in both fields was well above the dyke level, usually about 15 inches. Just as at Orgarswick it was noticeable that the soil of the poor field was more difficult to bore out and felt more compact than that on the good field; in consequence the falls of rainwater took a longer time to get away. Thus an inch of rain falling on June 3rd and 4th raised the water table in both cases by an inch, but on the succeeding day (June 5th) the water below the fatting field had fallen to its previous level, whereas it now stood 2 inches higher in the non-fatting field. On the 6th and 7th there was still a comparative difference of half an inch as the result of the rainfall, and similar results were obtained on other occasions.

Turning to the amount of available nitrogen as nitrate and ammonia it is found as at Orgarswick that during the spring months there is always a much higher proportion of these constituents in the soil of the

fatting field, though the total nitrogen of the two soils is alike, and the increased growth on the fatting field must always be reducing the amount of nitrate. In September the proportions are reversed. As at Orgarswick the rate of nitrate production measured in the laboratory is greater in the soil of the fatting field than in the other.

### *Westbroke.*

At this centre there is an essential difference in type between the soils of the good and the bad field. The good field is a light loam passing into sand in the 4th foot, the poor field is a heavy clay for at least 4 feet down. But the clay properties must be considerably modified by the large amount of organic matter, for neither in temperature nor in water content is there any great difference between the two soils. The adjoining Lydd field, representing a different type and carrying a wholly different herbage, is almost a pure sandbank, and it should be noticed that the Westbroke fatting field passes into very similar sand in its 4th foot. This part of the Marsh is much older than the land at the other two stations. There is evidence in fact that the town of Lydd was built upon a sandbank stretching across the tidal estuary which now forms the Marsh, and that it was settled dry land in the earliest historic times when the area behind was still awash with the tides. There is rather more difference in moisture content than at Midley and Orgarswick especially after a spell of dry weather, the non-fatting field beginning to dry out while the surface soil of the fatting field remains moist despite its lighter texture. The soil of the poor Lydd field generally contained a smaller proportion of water near the surface. The temperature differences of the three fields are again insignificant though the non-fatting field is always slightly warmer than the fatting field. The yield of grass is much the highest from the fatting field and lowest in the Lydd field, especially in the early part of the season. The chemical analyses (Table 6) show no more than before, the only significant differences being a slightly greater amount of nitrogen and a distinctly greater. amount of phosphoric acid in the soil of the fatting field. As before, the striking difference is seen in the quantity of available nitrogen during the earlier part of the season ; there is always much more nitrate and more ammonia in the soil of the fatting field and laboratory measurements showed a higher rate of nitrate production. The poor Lydd field also contains less nitrate during the early part of the season, but the differences are reversed from June onwards ;

the proportion of ammonia in the soil of this field is also comparatively high.

Considering the results of all three stations together no very distinct factors emerge to account for the differences in the amount and feeding value of the herbage. The results afforded by the mechanical analyses are inconclusive; at Orgarswick, where the heaviest soils occur, the good and the bad field show almost identical composition. At Midley the good field possesses a lighter surface soil, but the difference disappears below the top 12 inches. At Westbroke the good field possesses a very light loamy soil passing into sand, whereas the poorer field possesses 4 feet of a distinctly heavy soil resting on shingle and the bad Lydd field is a sand rather lighter than the good field. Mechanical analysis, however, loses much of its value on pasture soils where the properties are determined more by the organic matter than by the mineral fractions; in these soils the moisture content of the pairs of fields at different times of the season shows no such uniform differences as would be expected between light and heavy soil but varies sometimes in one direction and sometimes in the other. As a rule the good fields do not show the effect of drought quite so quickly as the others. Temperature differences are small and again sometimes in the one sense and sometimes in the other. The good soils of Orgarswick and Midley agree however in being a trifle more elevated than the poor soils, and the water table is to an even greater degree further from the surface in the good soils. Again the poor soils offer more resistance to the movements of water and the rainfall does not drain away so readily. But while these differences may be observed in the pairs and are possibly of considerable significance, we are unable to reduce them to any expression that holds generally.

The chemical analyses do show certain constant differences; on the whole there is more total nitrogen in the soil of the good fields, which have also a considerable superiority as regards phosphoric acid. It should be noted that this superiority is only evident in the total phosphoric acid and not in the citric acid soluble phosphoric acid, which is always extremely low. Doubtless the citric acid method as a measure of available phosphoric acid cannot be held to apply to pasture soils.

Finally, and here is the most significant difference that chemical analysis reveals, the amount of nitrates and ammonia in the good soils is always far above the quantities found in the poor soils in the early part of the year. Here is probably the causal factor which accounts

both for the greater amount of growth on the fatting fields and its green broad leafy character. But accepting this more rapid production of available nitrogen compounds as the determining factor giving rise to the herbage, it is still impossible to see from the other determinations made why the formation of available nitrogen compounds should be more rapid in the one case than in the other. The difference may lie in the nature of the organic matter of the soil.

### Floral type on other soils.

From the preceding results it has become clear that the floral type at any one place is almost identical on the fatting and non-fatting field,

TABLE 9. *Rich Soils, well provided with moisture.*
*Season 1909. Kent Coast.*

| | Romney Marsh | | Richborough | | | | |
| | Midley | Westbroke | | | Herne | Chislet | Graveney |
| | Fatting | Fatting | Good | Black leg | | | |
|---|---|---|---|---|---|---|---|
| Lolium perenne ......... | 55·86 | 50·48 | 36·22 | 43·64 | 46·57 | 43·99 | 25·44 |
| Agrostis vulgaris & alba | 4·22 | 13·51 | 1·22 | 0·50 | 1·12 | 0·87 | 3·20 |
| Cynosurus cristatus ... | 13·41 | 15·75 | 10·89 | 9·78 | 11·93 | 10·88 | 9·12 |
| Avena flavescens ......... | 8·48 | 0·85 | 15·04 | 13·27 | 1·96 | 15·28 | 8·76 |
| Hordeum pratense ...... | 1·69 | — | 19·33 | 13·05 | 3·04 | 11·54 | 6·92 |
| Phleum pratense ......... | 0·85 | — | 2·62 | 0·19 | 1·45 | 4·24 | 0·10 |
| Poa trivialis ............... | 6·01 | 1·34 | 1·11 | 4·55 | 1·36 | 6·76 | 4·26 |
| Holcus lanatus ......... | 0·91 | 10·65 | — | — | 5·72 | — | — |
| Dactylis glomerata ...... | — | — | 1·25 | — | — | 1·11 | — |
| Festuca ovina ............ | — | — | 6·80 | 4·44 | 0·12 | 1·40 | 9·31 |
| Alopecurus agrestis ... | — | — | — | — | — | — | 2·15 |
| Poa pratensis ............ | — | — | — | — | — | — | 0·23 |
| Sedges, &c. ............... | — | — | — | — | — | — | 15·52 |
| Leguminosae ............ | 7·65 | 4·44 | 4·76 | 8·12 | 23·77 | 2·68 | 12·52 |
| Miscellaneous ............ | 0·87 | 2·96 | 0·73 | 2·43 | 2·92 | 1·22 | 2·42 |

though the soil conditions may be similar as at Orgarswick or very different as at Westbroke. Thus it appears that the floral type is more influenced by local climate, situation and management than by soil, and this conclusion is strengthened by the fact, also brought out in Table 1, that it varies very considerably on the same field from year to year, and even from month to month. Table 9 gives the botanical analyses of the herbage taken at approximately the same date from a number of sheep pastures in the Kent and Sussex marshes. Speaking

generally the conditions are much alike and the soils possess a certain similarity due to their similar origin. All the samples possess certain factors in common, Rye Grass is the most abundant grass though it forms only 25 per cent. of the herbage at Graveney. Crested Dogstail also figures largely and is clearly a characteristic grass of the Southern sheep pastures. The leguminous plants, particularly white clover, are always well in evidence and as a rule more abundant in the grazing herbage than the analyses of these fully grown herbages would indicate. *Hordeum pratense* is sometimes abundant, being a characteristic grass of alluvial pastures. The weeds are never very abundant though the Graveney pastures contain a large proportion of sedges which do not figure in the other cases. Little idea can be formed from the analyses as to the feeding value of the different pastures. For example, of the two Richboro fields one is represented as being exceptionally good and producing fine wool and mutton; sheep from off this marsh are always of better repute in the market than those from Romney Marsh, being supposed to kill with more flesh in proportion to fat. The Black Leg field is much inferior, though the composition of the herbage is very similar and the two fields are under the same management, as again is the Chislet field which is good though not so good as the better Richboro field. Herne field is known to have been plough-land that was allowed to fall down many years ago. It will be noticed that this herbage has become very similar to that at Richboro and Chislet, places in the same general sweep of marsh. On these marshes there is more diversity of herbage than is usual on good fields, where as a rule there are only three or four dominant species. Speaking generally this Table may be taken to illustrate the fact that the floral type does vary from place to place with different conditions of situation and climate. We have already said that soil is not as a rule a very large factor in determining the floral type. Sometimes however it becomes the dominant factor, especially when it has been accompanied by different management. This is illustrated by the difference in composition of the herbage in Westbroke and the adjoining Lydd field where the soil is almost a pure sand. Climate and situation however are here identical and the differences, which as Table 1 shows persist right through the season, may be set down largely to the soils.

Lastly management is a potent factor in the floral type. Table 10 shows the botanical analyses from various other pastures, including two pairs of comparisons of adjoining good and poor fields. At Hope All Saints the soils are more nearly alike than at Westbroke (see Table 3),

the one field is a very good fatting pasture consisting mainly of Rye grass, the other field with a heavier soil shows every sign of neglect and mismanagement and the grass possesses much less feeding value. Arguing from the analogy of the identity of floral type on the good and bad Midley, Orgarswick and Westbroke fields, nothing but difference of management would seem to account for the difference in the herbage

TABLE 10.

| | 1909 | | 1909 | | 1879—1888 | 1909 |
|---|---|---|---|---|---|---|
| | Hope All Saints sheep pastures Romney Marsh | | Oving bullock pastures | | Market Harboro, Leicestershire bullock pastures | Beddingham (E. Sussex) |
| | Fatting | Non-fatting | Good old pasture | Poor pasture | | |
| Lolium perenne ............ | 81·18 | 25·55 | 32·90 | 5·45 | 27·40 | 48·76 |
| Agrostis vulgaris ......... | 0·55 | 10·04 | 0·75 | 1·74 | 13·30 | 8·51 |
| Cynosurus cristatus ...... | 6·58 | 13·32 | 3·51 | 3·06 | 5·95 | 5·55 |
| Avena flavescens............ | 1·06 | 14·16 | 2·41 | 6·96 | 1·17 | — |
| Hordeum pratense......... | 0·58 | 12·34 | 0·71 | 11·36 | 0·77 | 9·13 |
| Phleum pratense ......... | 0·47 | 0·96 | 7·54 | 0·50 | 3·25 | 13·9 |
| Poa trivialis.................. | 7·40 | 0·70 | 0·36 | 0·52 | 6·56 | 1·93 |
| Holcus lanatus ............ | — | 0·38 | 1·01 | 3·31 | 4·46 | 0·11 |
| Dactylis glomerata......... | — | 10·07 | 5·94 | 7·85 | 3·26 | 1·68 |
| Festuca ovina .............. | — | 0·81 | 14·22 | 10·63 | 1·31 | 0·22 |
| Anthoxanthum odoratum | — | — | — | — | 0·13 | ... |
| Bromus mollis ............ | — | 3·77 | — | 0·12 | — | — |
| Festuca pratensis ......... | — | — | 0·53 | 0·14 | 0·12 | — |
| Arrhenatherum avena-ceum...................... | — | — | — | 0·44 | — | ... |
| Agropyron repens ......... | — | — | — | 4·07 | — | 0·53 |
| Poa pratensis .............. | — | — | — | 1·30 | — | ... |
| Alopecurus pratensis ...... | — | — | — | — | 0·54 | ... |
| Alopecurus agrestis ...... | — | — | — | — | — | 1·81 |
| Poa annua ................. | — | — | — | — | 3·51 | — |
| Briza media.................. | — | — | — | — | 0·12 | — |
| Leguminosae .............. | 2·00 | 2·91 | 13·64 | 21·80 | 21·37 | 2·97 |
| Miscellaneous .............. | 0·14 | 4·96 | 29·24 | 20·60 | 2·79 | 4·84 |

of these two fields. Similarly on the Oving pastures soil and climate conditions are apparently identical, but the good field represents an old pasture which has apparently never been ploughed and will fatten 1¼ bullocks to the acre in a good season without any artificial food, whereas the poor pasture was allowed to tumble down about 40 years ago, and has been neglected ever since. This herbage possesses little feeding value, and hay made from the field has no strength and rarely

TABLE 11. *Percentage of fibre in dry matter of Romney Marsh grasses.*
Whole herbage.

| | | Orgarswick | | | | |
|---|---|---|---|---|---|---|
| | 1908 | 1909 | | | 1910 | |
| | | June 15 | July 22 | May | June | Sept. |
| Fatting grass, leafy habit ......... | 26·6 | 25·7 | 27·4 | 17·6 | 29·4 | 28·6 |
| Non-fatting grass, stemmy habit | 28·3 | 27·5 | 28·2 | 19·8 | 29·2 | 25·2 |

| | | Midley | | | Westbroke | | | |
|---|---|---|---|---|---|---|---|---|
| | 1909 | 1910 | | | 1909 | 1910 | | |
| | July 5 | May | June | Sept. | July 14 | May | June | Sept. |
| Fatting grass, leafy habit ......... | 28·7 | 16·1 | 31·7 | — | 26·1 | 17·8 | 32·9 | 26·0 |
| Non-fatting grass, stemmy habit | 28·2 | 16·7 | 32·4 | 24·8 | 27·3 | 17·6 | 30·9 | 26·6 |
| Lydd poor field | | | | | 29·1 | 18·4 | 27·1 | |

Separate species, 1909.

| | Lolium | Cynosurus | Avena | Agrostis | Hordeum | Poa | Holcus | Leguminosae | Miscellaneous |
|---|---|---|---|---|---|---|---|---|---|
| *Midley :—* | | | | | | | | | |
| Fatting ... | 25·9 | 28·8 | 32·1 | 27·1 | 27·2 | 29·7 | — | — | 22·6 |
| Non-fatting | 27·7 | 27·6 | 28·1 | 27·7 | 26·9 | 26·0 | — | 16·2 | 29·4 |
| *Westbroke:—* | | | | | | | | | |
| Fatting ... | 29·2 | 36·1 | 30·7 | 26·3 | — | 29·6 | 29·9 | 19·0 | 26·7 |
| Non-fatting | 28·8 | 31·4 | — | 24·8 | — | 32·4 | 30·5 | 18·5 | 34·5 |
| Lydd poor | 30·4 | 31·2 | 28·9 | 27·7 | 33·2 | — | 19·7 | 16·6 | 29·6 |
| *Orgarswick, June 15:—* | | | | | | | | | |
| Fatting ... | 26·5 | 25·2 | 24·8 | 23·6 | 22·4 | 24·0 | — | — | 20·7 |
| Non-fatting | 25·4 | 24·3 | 24·7 | 24·4 | 24·2 | 26·1 | — | — | 22·6 |
| *Orgarswick, July 22:—* | | | | | | | | | |
| Fatting ... | 27·7 | 24·6 | 24·4 | 23·8 | 27·9 | 25·6 | — | — | — |
| Non-fatting | 28·2 | 26·5 | 26·3 | 26·3 | 31·2 | 24·0 | — | — | — |

Mean of samples from a number of fields, 1909.

| Lolium | Cynosurus | Avena | Agrostis | Hordeum | Poa | Holcus | Phleum | Leguminosae | Miscellaneous |
|---|---|---|---|---|---|---|---|---|---|
| * 30·6 | † 32·1 | ‡ 30·2 | ‡ 28·6 | § 30·1 | § 28·0 | ‖ 25·4 | ‖ 28·5 | ¶ 22·8 | ¶ 28·2 |

\* 21 fields.  † 20 fields.  ‡ 19 fields.  § 17 fields.  ‖ 7 fields.  ¶ 16 fields.

gets hot in the stack. The analyses of the Leicestershire pastures were made in the Rothamsted Laboratory in the years 1879, 1880, 1882, 1888. It is interesting to see that the floral type is very similar to that at Oving. The Beddingham pasture on the Ouse marshes is stocked with sheep in the spring, then laid up for hay and finally grazed with cattle. The notable feature is the large proportion of Timothy, a grass usually found in only small proportions in the other pastures. Speaking generally this Table illustrates the fact that management may be a very potent factor in determining the floral type. Taking all these examinations of herbage together we can only conclude that the feeding value of a pasture is largely independent of the floral type, and that whether any particular species of the grasses here considered will be good or poor food depends upon the soil and climatic conditions and particularly on the management.

### The chemical composition of the herbage.

Great differences in the habit of growth of the grasses from the good and poor field respectively led us to expect such corresponding differences in the chemical composition as would account for the variations in their feeding value. In the main this expectation was not realised. In the first place the stemmy appearance of the grass on the non-fatting field contrasts so markedly with the softer leafy appearance of the grass from the fatting fields that one expected they would show pronounced differences in the proportion of fibre. Table 11 shows that this is not the case, and that the whole herbage taken at various dates in 1909 and 1910 from the adjacent fields at Orgarswick, Midley and Westbroke contain practically identical amounts of fibre. In 1909 the mixed herbage was also sorted into its chief constituent species and analyses were made of this material representing one species only. Again no consistent differences were observed, nothing in fact to account for the superior feeding value of the samples from the fatting field. Nitrogen determinations were also made as shown in Table 12. This table does reveal certain constant differences, as the herbage from the fatting field almost invariably shows higher percentages of nitrogen, as indeed might have been expected from the higher nitrate content of the soil from the fatting fields. A few determinations were made of the proportion of nitrogen in the herbage which would be brought into a soluble condition by the action of pepsin, and by this method again certain small differences are revealed, for the herbage from the fatting fields always contains both a higher total amount of digestible nitrogen

TABLE 12. *Percentage of nitrogen in dry matter of Romsey Marsh grasses.*

| | 1908 | Orgarswick | | | | |
|---|---|---|---|---|---|---|
| | | 1909 | | 1910 | | |
| | | June 15 | July 22 | May | June | Sept. |
| Fatting grass...... | 1·80 | 3·21 | 2·96 | 2·96 | 2·49 | 2·20 |
| Non-fatting grass | 1·70 | 3·13 | 2·77 | 2·86 | 2·48 | 2·24 |

| | Midley | | | | Westbroke | | |
|---|---|---|---|---|---|---|---|
| | 1909 | 1910 | | | 1909 | 1910 | |
| | July 5 | May | June | Sept. | July 14 | May | June | Sept. |
| Fatting grass...... | 2·63 | 3·10 | 2·73 | — | 2·63 | 3·93 | 3·02 | 2·76 |
| Non-fatting grass | 2·68 | 2·99 | 2·80 | 2·30 | 2·14 | 3·65 | 2·14 | 2·14 |
| Lydd poor field | | | | | | 3·41 | | 2·31 |

Separate species, 1909.

| | Lolium | Cyno-surus | Avena | Agrostis | Hor-deum | Poa | Legu-minosae |
|---|---|---|---|---|---|---|---|
| *Midley :—* | | | | | | | |
| Fatting............... | 2·63 | 2·34 | 2·63 | 2·67 | — | 2·67 | 3·86 |
| Non-fatting ........... | 2·68 | 2·12 | 2·75 | 2·71 | 2·70 | 2·57 | 3·75 |
| *Westbroke :—* | | | | | | | |
| Fatting............... | 2·63 | 2·08 | 2·28 | 2·67 | — | 2·32 | 3·75 |
| Non-fatting ........... | 2·14 | 1·68 | — | 2·54 | — | — | 3·56 |
| Lydd poor ............ | 2·43 | 1·67 | 2·41 | 3·06 | — | — | 4·09 |
| *Orgarswick, June 15:—* | | | | | | | |
| Fatting............... | 3·21 | 3·04 | 3·42 | 3·48 | 3·34 | 3·02 | 4·09 |
| Non-fatting........... | 3·13 | 2·99 | 3·41 | 3·35 | 3·11 | 2·81 | — |
| *Orgarswick, July 22:—* | | | | | | | |
| Fatting............... | 2·96 | 2·84 | 3·38 | 3·40 | 2·79 | 3·31 | 3·93 |
| Non-fatting........... | 2·77 | 2·79 | 3·26 | 3·14 | 2·47 | 3·20 | — |

Mean of samples from 21 fields, 1909.

| Lolium | Cynosurus | Avena | Agrostis | Hordeum | Poa | Leguminosae |
|---|---|---|---|---|---|---|
| 2·09 | 1·79 | 2·23 | 2·34 | 2·14 | 2·34 | 3·36 |

and a higher proportion of the whole nitrogen in a digestible state. Thus the 1909 Orgarswick samples gave the following results:

| | Total N | N digested by pepsin | Digestible coefficient |
|---|---|---|---|
| June 15th | | | |
| Fatting | 3·21 | 2·75 | 85·5 |
| Non-fatting | 3·20 | 2·65 | 82·7 |
| July 22nd | | | |
| Fatting | 3·16 | 2·70 | 85·4 |
| Non-fatting | 2·87 | 2·34 | 81·6 |

Table 13 shows the amount and composition of the ash obtained from the mixed herbage of the three pairs of fields at various dates in 1908 and 1909. The proportion of ash bears no constant relation to the fatting qualities of grass, in fact the only constant difference that this analysis reveals is to show a slightly greater proportion of phosphoric acid in the soluble ash of the herbage from the fatting field, thus corresponding to the greater amount of phosphoric acid in the

TABLE 13. *Percentage of mineral constituents in*
*Romsey Marsh herbage.*

| | Composition of dry matter, per cent. | | | | | Composition of soluble ash, per cent. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ash | $P_2O_5$ | $K_2O$ | CaO | $Mn_3O_4$ | $P_2O_5$ | $K_2O$ | CaO | $Mn_3O_4$ |
| *Orgarswick.* 1908. | | | | | | | | | |
| Fatting grass | 11·22 | 0·64 | 4·37 | | | 8·11 | 55·18 | 7·70 | |
| Non-fatting grass | 10·59 | 0·44 | 3·21 | | | 7·53 | 54·78 | 8·28 | |
| June 15th, 1909. | | | | | | | | | |
| Fatting grass | 10·94 | 0·56 | 3·99 | 0·91 | 0·002 | 6·57 | 46·66 | 10·59 | 0·27 |
| Non-fatting grass | 12·71 | 0·58 | 4·12 | 1·54 | 0·006 | 6·41 | 45·41 | 16·97 | 0·75 |
| July 22nd, 1909. | | | | | | | | | |
| Fatting grass | 12·57 | 0·62 | 4·68 | 1·03 | 0·004 | 6·68 | 50·69 | 11·17 | 0·42 |
| Non-fatting grass | 13·75 | 0·63 | 3·86 | 1·01 | 0·010 | 6·60 | 40·11 | 10·52 | 1·01 |
| *Midley.* July 5th, 1909. | | | | | | | | | |
| Fatting grass | 11·33 | 0·54 | 3·70 | 1·07 | 0·003 | 6·45 | 44·49 | 12·82 | 0·40 |
| Non-fatting grass | 10·67 | 0·39 | 3·43 | 0·97 | 0·005 | 5·11 | 44·68 | 12·68 | 0·60 |
| *Westbroke.* July 14th, 1909. | | | | | | | | | |
| Fatting grass | 12·01 | 0·53 | 3·46 | 0·93 | 0·009 | 7·14 | 46·45 | 12·47 | 1·14 |
| Non-fatting grass | 11·01 | 0·45 | 3·11 | 0·86 | 0·002 | 6·34 | 43·44 | 12·01 | 2·47 |
| Very poor | 11·19 | 0·50 | 3·52 | 1·05 | 0·018 | 7·07 | 49·50 | 14·81 | 2·49 |

soil; this does not, however, perceptibly increase the phosphoric acid in the dry matter. It is perhaps worthy of notice that the oxide of manganese is always higher in the non-fatting herbage, as it is almost invariably in the soil of the non-fatting field. We possess so little knowledge of the function of manganese in either plant or animal life that it is impossible to say whether this fact possesses any significance. Table 14 shows a similar state of determinations of the composition of the mixed herbage from the other marshes, again including three pairs of adjoining good and poor fields. The results are equally inconclusive;

TABLE 14. *Composition of dry matter, whole herbage.*

| | Fibre | Nitrogen | Ash | P$_2$O$_5$ | K$_2$O | CaO | Oil |
|---|---|---|---|---|---|---|---|
| Beddingham .............. | 31·4 | 1·86 | 10·42 | 0·60 | 3·08 | 0·99 | 7·6 |
| Richborough (Good) ...... | 32·6 | 2·07 | 11·50 | 0·46 | 2·89 | 1·03 | 8·8 |
| Richborough (Black leg)... | 30·9 | 2·28 | 13·08 | 0·59 | 3·04 | 1·24 | 2·5 |
| Chislet ..................... | 31·0 | 2·21 | 14·13 | 0·46 | 3·48 | 1·01 | |
| Herne ..................... | 33·1 | 2·00 | 11·56 | 0·38 | 2·35 | 0·95 | |
| Graveney ................... | 29·7 | 2·15 | 9·74 | 0·44 | 2·05 | 0·80 | 2·3 |
| Sandhurst, Kent:— | | | | | | | |
| Good pasture ........... | 33·6 | 1·75 | 11·86 | 0·64 | 2·58 | 1·05 | |
| Poor pasture ........... | 34·7 | 1·97 | 10·29 | 0·55 | 2·41 | 1·35 | |
| Oving :— | | | | | | | |
| Good pasture ........... | 29·9 | 2·00 | 9·05 | 0·41 | 1·64 | 1·43 | |
| Poor pasture ........... | 30·0 | 1·84 | 8·73 | 0·46 | 1·52 | 1·30 | |
| Hope All Saints :— | | | | | | | |
| Good pasture ........... | 33·2 | 2·64 | 11·20 | 0·58 | 4·09 | 0·87 | |
| Poor pasture ........... | 31·9 | 2·04 | 9·23 | 0·40 | 2·97 | 0·70 | |

there is a general tendency for the grass of the best fields to show a slightly higher proportion of nitrogen and phosphoric acid, but these differences are never such as can be taken to account for the differences in feeding value. The only general conclusion one can draw is that the method of food analysis as ordinarily practised gives no measure of the feeding value of such material as grass. It fails to reveal anything to correspond to the very marked differences in habit of the fatting and non-fatting grasses, and none of the results can be interpreted so as to show which of the grasses were poor and which valuable food.

## CONCLUSIONS.

(1) The feeding value of pasture grass is determined not only by the floral type (*i.e.* the botanical composition of the herbage) but also by the habit of growth.

(2)   The floral type is determined by climatic factors—temperature, and the supply of air and of water to the roots—the reaction of the soil and the treatment of the grass, but is not necessarily affected by variations in the amount of nitrogenous plant food present.

(3)   The habit of growth is governed by a different set of factors more difficult to ascertain. In the cases dealt with in this paper the most important appeared to be the supply of nitrates and ammonia in the soil, *i.e.* the ease of decomposition of the organic matter; the supply of phosphate was also an important factor.

(4)   Thus floral type and habit of growth are independent. Cases are described in this paper where the general soil conditions and floral type persist over two adjoining fields, but the habit of growth and the feeding value of the grass are very different.

(5)   In attempting to ameliorate a pasture it is necessary to ascertain whether its poverty is due to bad floral type or to habit of growth. Mere casual inspection is insufficient to determine differences in type because a tendency to flower may make one species appear much more prominent than it really is. Thus the percentage of buttercups was found to be the same in two fields but appeared to be much higher in the field where it produced flowers than in the other where it did not.

(6)   In the cases examined a leafy habit of growth obtained in the fatting fields, and a stemmy habit in the poorer fields, the floral type being, as already stated, constant. Although the difference in feeding value was known to be great the differences revealed by the ordinary methods of chemical analysis were very small. The ordinary methods are clearly inadequate for dealing with pasture grasses.

(7)   The soils of the fatting fields possessed no constant properties revealed by the ordinary chemical or mechanical analyses. Their striking characteristic was the high rate at which nitrates were produced, they also contained a relatively large amount of total phosphoric acid. Experiments on the spot showed that they had a somewhat better texture than the soils of the non-fatting fields, allowing excess of water more readily to drain away, and retaining moisture better during dry weather, but this property could not be correlated with the mechanical composition of the soil. Soil analysis does not give as clear indications with pasture soils as it does with arable soils.

# THE ACTION OF RENNET ON MILK.

By M. NIERENSTEIN and JESSIE STUBBS.

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University of Bristol.*)

DURING the renneting process in the manufacture of Cheddar cheese a retardation of the time of coagulation has often been noticed though the acidity had reached the required strength before the rennet was added to the milk. The cause or causes of this retardation are of great technical importance as they lead to infection of the milk with consequent production of taints. As the two main factors of coagulation are (i) the acidity of the milk when renneted, and (ii) the calcium salts of the milk, we approached the inquiry from these two points and tried to answer the following questions :—

(1) Is *all* the acidity of the milk produced on standing due to the formation of lactic acid?

(2) Would it be possible to produce a real *Cheddar Cheese Curd* if to the milk the necessary amount of lactic was added before renneting?

(3) How far is the coagulation time retarded on dialysis of the calcium salts of the milk?

As regards the first question our inquiry has led us to believe that the total acidity is not actually due to lactic acid, and this is brought out in Table I which gives the amounts of lactic sugar in milk at different times

## TABLE I.

| Time | Lactose | Lactic acid | | |
|---|---|---|---|---|
| | | Found | Increase of acidity | |
| | | | Found | Calc. for max. 4 mols |
| hours | grams | grams | grams | grams |
| 0 | 3·762 | 0·926 | — | — |
| 6 | 3·224 | 1·723 | 0·796 | 0·538 |
| 19 | 2·965 | 2·764 | 1·838 | 0·797 |
| 27 | 2·249 | 3·286 | 2·360 | 1·513 |

together with the acidity.   The figures are expressed for 100 c.cs. milk. The sugar estimations were carried out according to Ritthausen[1], and the acidity estimated by titration with N/10 caustic soda, using phenol phthalein as an indicator.

TABLE II.

*Temperature:  33—35° C.*

| Time | Acidity expressed in N/1000 KOH | Increase of acidity |
|---|---|---|
| mins. | c.cs. | c.cs. |
| 0 | 0·4 | — |
| 30 | 11·40 | 11·0 |
| 60 | 17·80 | 17·4 |
| 90 | 24·60 | 24·2 |
| 120 | 34·20 | 33·8 |
| 150 | 41·70 | 41·3 |
| 180 | 68·40 | 68·0 |
| 210 | 87·7 | 87·3 |
| 240 | 94·8 | 94 4 |
| 270 | 102·6 | 102·2 |

TABLE III.

*Temperature :  24° C.*

| Time | Acidity expressed in N/1000 KOH | Increase of acidity |
|---|---|---|
| mins. | c.cs. | c.cs. |
| 0 | slightly alkaline | — |
| 30 | 8·8 | — |
| 60 | 14·8 | 6·0 |
| 90 | 23·4 | 14·6 |
| 120 | 34·7 | 25·9 |
| 150 | 43·1 | 34·3 |
| 180 | 64·3 | 55·5 |
| 210 | 78·4 | 69·6 |
| 240 | 94·6 | 85·8 |

These results, which are only one instance taken from three similar experiments, obviously indicated that the increase of the acidity of milk was not due to the formation of lactic acid only, and this was confirmed by the following experiments.   To a solution of caseinogen made as neutral as possible by Hammarsten's method, rennet was added (3 g. casein-ogen in 50 c.cs. Ca(OH)$_2$ and phosphoric acid and 0·2 c.c. of a 1 per cent.

[1] Ritthausen, *Journ. f. prakt. Chemie*, II. **16**. 329 [1877].

solution of rennet were used). The increase of acidity was estimated for 5 c.cs. with N/1000 KOH solution and phenol phthalein used as an indicator. The estimations were made at 33—35° C. and 24° C. The results are expressed in Tables II and III.

We then further studied the effect of rennet in the presence of lactic acid in order to see whether this acid does or does not stimulate a higher production of acidity during the action of rennet on caseinogen. To a caseinogen solution we therefore added 25 c.cs. of a 1/40 per cent. lactic acid solution and carried out the estimation as before. Table IV gives the results.

TABLE IV.

*Temperature:* 24° C.

| Time | Acidity expressed in N/1000 KOH | Increase of acidity |
|------|-------------------------------|---------------------|
| mins. | c.cs. | c.cs. |
| 0 | 44·6 | — |
| 30 | 64·8 | 20·2 |
| 60 | 79·2 | 34·6 |
| 90 | 94·7 | 50·1 |
| 120 | 127·2 | 82·6 |
| 150 | 147·4 | 102·8 |
| 180 | 189·7 | 145·1 |

These results show that rennet is capable of producing acidity from caseinogen and that this process is stimulated by the addition of lactic acid. In the present position of the investigation it is difficult to understand how the acidity is actually produced, and further work on these lines must be carried out before we are in a position to offer a definite explanation.

An increase of acidity having been fully established it was thought possible that lactic acid might be used as a "starter" in the renneting of milk, since we had found that it apparently acted as a stimulant in the production of acidity. The advantages of such a material as a "starter" are obvious. To 10 gallons of fresh milk 10 *c.cs. pure Kahlbaum's lactic acid* and 23 c.cs. rennet were added. At the time of renneting the acidity of the milk was approximately 0·14 per cent., which is the required acidity in the renneting of milk for Cheddar cheese. Soon after the rennet was added the milk coagulated and formed a curd consisting of *non-adhering parts quite unlike the curd formed under ordinary conditions.* At that moment the acidity was 0·17 per cent. and did not

change for 9 hours, which is also quite abnormal. It afterwards pro-gressed slowly in 12 hours to 4·7 per cent. and in 13 hours to 7·2 per cent., whereas under normal conditions this acidity would have been reached in 5—7 hours. The cheese thus obtained consisted of a hard crummy mass and was certainly of a very inferior type. We believe this was due to the fact that during the process the caseinogen had been precipitated out by lactic acid and not transformed into casein. In the light of our previous experiments it must be concluded that the acidity of milk before renneting is certainly not due to lactic acid *only*, for were this acid present in the milk to the same extent when usually renneted it would precipitate out the caseinogen and hence prevent its transformation into casein.

As the retardation of the coagulation could have been explained by the fact that the milk did not contain enough calcium, we estimated the coagulation time of milk before and after dialysis. However, as it will be seen from Table V, it is quite impossible to conclude that this can be explained on such lines. The calcium estimations were carried out in the following way: the milk or dialysed milk was evaporated to dryness and the calcium estimated in the ash by precipitating with oxalic acid, washing the precipitate carefully and finally transfer-ring to a beaker by means of a jet of water and titrating with potassium permanganate in the presence of sulphuric acid.

TABLE V.

|  | Non-Dialysed Milk | | | Dialysed Milk | | |
|---|---|---|---|---|---|---|
|  | Acidity | Calcium in 10 c.cs. | Time of coagn. of 25 c.cs. | Time of Dialysis | Calcium in 10 c.cs. | Time of coagn. of 25 c.cs. |
|  | per cent. | gms. CaO | mins. | hours | gms. CaO | mins. |
| 1 | 0·21 | 0·0116 | 31 | 4 | 0·0102 | 57 |
| 2 | 0·22 | 0·0112 | 25 | 4 | — | 38 |
| 3 | 0·16 | 0·0100 | 30 | 4 | 0·0098 | 46 |
| 4 | 0·16 | 0·0112 | 27 | 4 | 0·0102 | 34 |
| 5 | 0·19 | 0·0110 | 20 | 4 | 0·0098 | 10 |
| 6 | 0·16 | 0·0118 | 31 | 4 | 0·0108 | 32 |
| 7 | 0·16 | 0·0116 | 20 | 4 | 0·0088 | 25 |
| 8 | 0·17 | 0·0110 | 22 | 19 | 0·0082 | 56 |
| 9 | 0·16 | 0·0116 | 30 | 19 | — | — |
| 10 | 0·17 | — | 23 | 23 | 0·0082 | 50 |

If we analyse the results in Table V we find that there is no relationship between the length of the time the dialysis was allowed to

proceed and the loss of calcium, and that there is further no relationship between the loss of calcium and the retardation of the time of coagulation.

Though this investigation does not in any way throw light on the reason of the retardation of the coagulation, it has at any rate shown that this is not entirely due to the acidity or to the calcium salts of the milk as is usually assumed.

### Conclusions.

(1)  The acidity of milk is not due entirely to lactic acid, but partly to some product produced from caseinogen.

(2)  Pure lactic acid cannot be used as a starter, though it stimulates the production of acid from caseinogen.

(3)  The retardation of the time of coagulation with rennet is not entirely dependent on the calcium salts.

# DISTRIBUTION OF THE NITROGEN OF WHEAT BETWEEN THE FLOUR, BRAN AND SHORTS.

BY J. E. GREAVES AND ROBERT STEWART.

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IN the work which has been carried on by the chemical department of this station on the milling qualities of wheat[1], there has accumulated some very interesting and important data on the distribution of the nitrogen of wheat between the various milling products, and it is the purpose of this paper to consider the summarised results from this work.

The wheat was all passed through a fanning mill which removed all straw, chaff, various kinds of weed seeds, and most other constituents foreign to the wheat, after which it was passed through the scourer, which loosened and removed all impurities not previously removed, and also removed the fine hairs at the end of the berry and a very small portion of the outer covering of the wheat kernel. It was then milled in one of the small experimental mills built by the Allis-Chalmers Company of Milwaukee. The mill is provided with two sets of seven-inch rollers—one corrugated, the other smooth. The sifter, placed on a frame between the rollers, is provided with the requisite number of sieves. These sieves are covered with bolting silk, which is of the standard grades used in large flour mills. The mill is fitted with a feed adjustment so that the feeding can be nicely regulated. The products from the rollers are caught in drawers placed beneath the rollers; from these drawers they are transferred to the sifter. In order to facilitate this transfer without loss, a flat-bottom scoop is used. The drawers are brushed with a three-inch paint brush. This brush is also used in cleaning the mill after the milling of each sample. In order to have something to receive the various products milled, and that they may be transferred to the scale without loss, three pans were made. These were flat on the bottom and three sides, while the fourth side was drawn out into a funnel.

[1] Robert Stewart; and J. E. Greaves, *Utah Exp. Sta. Bul.* 103 and unpublished data.

The sample of wheat used in milling weighed about thirty pounds. It was run through the mill with the corrugated rollers set just close enough to mash the grain. The crushed product was then put into the sieves. The sieves were arranged in the following order: 1A uppermost, 2A next, and 3A on the bottom. No. 3A was made of No. 10 XX Shindler bolting silk. No. 2A was made of No. 30 gauze, while No. 1A was made of No. 20 wire screen. After all the finer material was sieved out, that remaining on sieve 1A was again run through the corrugated rollers, the rollers being closer together; again sieved as before and that which now remained on sieve 1A was set aside and weighed as finished bran. The products from sieves 2A and 3A were then passed through the smooth rollers and again sieved. That which still remained on sieve 2A was mixed with that taken from 1A and the whole recorded as bran. That which remained on sieve 3A was weighed and recorded as shorts, while all that passed through 3A was recorded as flour. The flour is, therefore, that part of the grain which, on being passed through the corrugated and smooth rollers, will pass through No. 10 XX Shindler bolting silk.

The various milled products were weighed on a set of beam balances, which were sensitive, with the load used, to within five grams. With this mill it is possible, with care, to mill a sample of wheat with very little loss, as is well shown by the fact that the average of the milling products in the analysis recorded below is 99·99 per cent. of the wheat. In fact, the error was usually less than 1 per cent.

The nitrogen was determined by the Kjeldahl method and all determinations were made in duplicate, and if there was not a close agreement, the determinations were repeated. The nitrogen, multiplied by 5·7, is reported as protein. The moisture was determined by heating about 3 grams of the products in a water oven for twelve hours.

All determinations were calculated to dry basis. As an average of 255 milling tests made on 58 varieties of wheat, the milling products were: flour, 68·10 per cent.; bran, 22·89 per cent.; shorts, 9·03 per cent. These factors, together with the percentages of nitrogen actually found in the grain and milled products, have been used to calculate the amount of protein (N × 5·7) which would actually occur in 100 pounds of the wheat and in the flour, bran and shorts resulting from the same, and also the percentage distribution of the protein of the grain between these different products.

Below is given the summarised results from 222 determinations on 42 varieties:

| Variety | No. of tests | Protein in 100 pounds of grain lbs. | Protein in milling products from 100 pounds of grain | | | Per cent. of protein of grain recovered in | | | Total °/o |
|---|---|---|---|---|---|---|---|---|---|
| | | | Flour lbs. | Bran lbs. | Shorts lbs. | Flour °/o | Bran °/o | Shorts °/o | |
| Nicaragua | 2 | 18·33 | 11·04 | 4·98 | 1·81 | 60·20 | 27·19 | 9·87 | 97·26 |
| New Zealand | 27 | 17·71 | 10·27 | 4·62 | 1·76 | 58·01 | 26·09 | 9·94 | 94·04 |
| White Club | 4 | 17·68 | 10·24 | 4·92 | 1·79 | 58·02 | 27·83 | 10·12 | 95·97 |
| Targova | 2 | 17·53 | 11·53 | 4·81 | 1·72 | 65·78 | 27·44 | 9·81 | 103·03 |
| Wellman's Fife | 4 | 17·45 | 11·16 | 4·61 | 1·65 | 63·93 | 26·42 | 9·46 | 99·81 |
| Salzier's Assiniboia Fife | 4 | 17·42 | 11·18 | 4·26 | 1·55 | 64·18 | 25·91 | 8·90 | 98·99 |
| Mohamed Ben Bachir | 9 | 17·27 | 11·32 | 4·40 | 1·57 | 65·56 | 25·48 | 9·09 | 100·13 |
| Currell | 2 | 17·14 | 9·88 | 4 97 | 1·69 | 57·64 | 29·00 | 9·86 | 96·50 |
| Crimean | 12 | 16·85 | 10·42 | 4·82 | 1·84 | 61·84 | 28·60 | 10·94 | 101·38 |
| Servian | 2 | 16·68 | 9·95 | 4·76 | 1·65 | 59·65 | 28·54 | 9·90 | 98·09 |
| Winter La Salle | 26 | 16·65 | 10·45 | 4·46 | 1·60 | 62·76 | 26·79 | 9·61 | 99 16 |
| Turkey | 17 | 16·57 | 10·19 | 4·45 | 1·52 | 61·46 | 26·84 | 9·16 | 97·46 |
| Romanow | 3 | 16·57 | 10·40 | 4·34 | 1·61 | 62·76 | 26·19 | 9·71 | 98·66 |
| Pellissier | 3 | 16·57 | 10·49 | 4·32 | 1·54 | 63·30 | 26·07 | 9·30 | 98·67 |
| Jap. Sq. Head | 2 | 16·57 | 9·42 | 5·12 | 1·75 | 56·84 | 30·89 | 10·56 | 98·29 |
| Odessa | 8 | 16·40 | 10·05 | 4·47 | 1·64 | 61·28 | 27·26 | 10·00 | 98·54 |
| Adjini | 2 | 16·39 | 10·73 | 4·55 | 1·58 | 65·47 | 27·76 | 9·64 | 102·87 |
| Ghirka | 4 | 16·35 | 9·82 | 4·82 | 1·70 | 60·06 | 29·48 | 10·39 | 99·93 |
| Beloglina | 6 | 16·35 | 10·48 | 4·69 | 1·65 | 64·10 | 28·68 | 10·09 | 102·87 |
| Medeah | 3 | 16·31 | 10·74 | 3·97 | 1·45 | 65·56 | 24·34 | 8·89 | 98·79 |
| Blue Stem | 7 | 16·28 | 9·78 | 4·61 | 1·62 | 60·08 | 28·32 | 9·95 | 98·35 |
| Hungarian | 4 | 16·26 | 10·22 | 4·76 | 1·63 | 62·86 | 29·27 | 10·03 | 102 16 |
| Ulta | 2 | 16·26 | 9·87 | 4·46 | 1·57 | 60·70 | 27·43 | 9·66 | 97·79 |
| Richi | 3 | 16·20 | 10·58 | 4·05 | 1·50 | 65·32 | 25·00 | 9·26 | 99·58 |
| Zimmerman | 5 | 16·18 | 9·10 | 4·94 | 1·75 | 56·24 | 30·53 | 10·82 | 97·59 |
| Red Chaff | 8 | 16·18 | 10·42 | 4·91 | 1·57 | 64·41 | 30·35 | 9·70 | 104·46 |
| Whitington | 2 | 16·10 | 10·17 | 4·49 | 1·63 | 63·18 | 27·98 | 10·13 | 101·2·) |
| Kharkov | 2 | 16·04 | 9·99 | 4·51 | 1·71 | 62·28 | 28·12 | 10·66 | 101·06 |
| Black Don | 3 | 15·97 | 10·00 | 4·55 | 1·45 | 62·58 | 28·47 | 9·08 | 100·13 |
| Weissenberg | 2 | 15·64 | 9·47 | 4·57 | 1·51 | 60·55 | 29·24 | 9·66 | 99·45 |
| Kofod | 6 | 15·61 | 9·82 | 4·69 | 1·53 | 62·91 | 30·04 | 9·80 | 102·75 |
| Missouri | 2 | 15·61 | 10·11 | 4·45 | 1·54 | 64·77 | 28·51 | 9·86 | 103·15 |
| Theiss | 2 | 15·53 | 9·95 | 4·31 | 1·57 | 64·08 | 27·75 | 10·11 | 101·94 |
| Alberta Red | 2 | 15·51 | 9·80 | 4·31 | 1·59 | 63·18 | 27·78 | 10·25 | 101·21 |
| Bulgarian | 2 | 15·46 | 9·21 | 4·34 | 1·56 | 59·58 | 28·07 | 10·09 | 97·74 |
| California Gem | 2 | 15·39 | 8·87 | 4·62 | 1·75 | 57·64 | 30·04 | 11·37 | 99·05 |
| Roumanian | 6 | 15·34 | 9·70 | 4·58 | 1·57 | 63·24 | 29·86 | 10·23 | 103·33 |
| Bosnian | 2 | 15·31 | 9·50 | 4·26 | 1·54 | 62·04 | 27·83 | 10·06 | 99·93 |
| Malakof | 2 | 15·12 | 9·58 | 4·40 | 1·61 | 63·36 | 29·11 | 10·65 | 103·12 |
| Gold Coin | 6 | 15·11 | 8·75 | 4·27 | 1·50 | 57·91 | 28·26 | 9·92 | 96·09 |
| Sonora | 8 | 14·95 | 9·78 | 4·07 | 1·39 | 65·41 | 27·24 | 9·30 | 101·95 |
| White Australian | 2 | 14·16 | 8·13 | 4·64 | 1·60 | 57·42 | 32·77 | 11·30 | 101·49 |
| Average | | 16·26 | 10·06 | 4·55 | 1·61 | 61·87 | 27·98 | 9·92 | 99·77 |

An examination of the above table reveals the following facts:

One hundred pounds of wheat with high nitrogen content such as Targova yielded 11·53 lbs. of protein in the flour obtained from it, while a low protein wheat such as White Australian yielded only 8·13 lbs. of protein, there being a difference of 3·5 lbs. of protein in the flour yielded by 100 lbs. of the two varieties of wheat. The per cent. of nitrogen in the wheat is not necessarily an index of the amount of nitrogen which appears in the flour, e.g., Nicaragua contained 18·33 per cent. nitrogen in the wheat, while Targova contained but 17·53 per cent., yet the latter yielded 0·5 lbs. more protein in the flour actually obtained than did the former. Gold Coin and Sonora contained nearly the same per cent. of nitrogen in the wheat (15·11 and 14·95 respectively), yet the latter yielded over 1 lb. more of nitrogen in its flour than did the former. It is further seen that the per cent. of the total protein of the wheat which was recovered in the flour varied from 56·84 in the case of Jap. Sq. Head to 65·5 in the case of Mohamed Ben Bachir. The low protein wheats yielded just as high a proportion of their nitrogen in the flour as did the richer protein wheats.

## SUMMARY.

The per cent. of nitrogen in a wheat is no direct index of the amount which its flour will contain. The per cent. of a wheat's protein recovered in the flour varied from 56·84 per cent. to 65·56, while the per cent. of protein occurring in the bran varied between 25 per cent. and 32·77 per cent., and that occurring in the shorts from 8·90 to 10·82 per cent. As an average of these determinations, the protein of the wheat was divided between the flour, bran and shorts in the proportion of 61·87 per cent., 27·98 per cent., and 9·92 per cent. respectively.

# AN INVESTIGATION CONCERNING THE FOOD OF CERTAIN BIRDS.

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## PART I. GENERAL.

THE investigation to be described was instigated by Professor Wood and Mr Warburton of the School of Agriculture, Cambridge, in the beginning of the year 1911. Its object was to determine whether or not certain birds were harmful to agriculture; as some naturalists and others considered them to be distinctly beneficial, while farmers were complaining of the damage done to their crops.

The first investigation concerning the food of birds in this country was made by Gurney and Russell (5)[1], who in 1885 examined the crops of sparrows killed at all seasons of the year. Since that time there have been comprehensive investigations by Gilmour (4) on wood pigeons, rooks and starlings; by Thorpe and Hope (19) on the black-headed gull; by Newstead (12) on the food of many species; and by Collinge (2 and 3) on the rook and starling.

The method adopted in this investigation was the examination of the stomach contents, together with a collection of field notes concerning each bird. The importance of this combination has been emphasized by Judd (9) and Palmer (14), who have also put forward another method; that of experiment on birds lately captured. The latter however has not been attempted in this investigation.

The various points taken into consideration when beginning were as follows :—

(1) That the examination of the stomach contents ought to be continued throughout the year, as the conditions (harvest, seed-time, ploughing, etc.) would vary considerably.

(2) That the district from which the birds to be investigated came should be extensive but not too wide. If all were taken from a small area, local conditions would be too prominent; whereas, if birds were

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[1] See List of References at end of this article.

obtained from the whole of Great Britain, the variations in climate (and consequently in dates of seeding and harvest) would prevent a clear idea being obtained of the changes in the food materials that occurred from month to month.

(3) That field notes ought to be taken when the birds were killed, the following facts being mentioned: (*a*) name and address of sender, (*b*) date and time of day, (*c*) exact locality in which the bird was killed, (*d*) weather, on account of its influence on insect life; (*e*) special notes, if the bird was doing any particular injury.

With the co-operation of many members of the Norfolk and Norwich Naturalists' Society, as well as others resident in Norfolk, Essex and Cambridgeshire, this object has been carried out.

I have here to express my sincere thanks to all those who sent birds and especially to Mr J. A. Christie, who sent me a large number.

The following is a list of the names of those who have sent birds:—
*Norfolk*:—Messrs J. H. Bugden, Suffield—E. Case, Kettlestone—J. A. Christie, Framingham Pigot—J. H. Gurney, Keswick—Q. E. Gurney, Northrepps—B. Hammond, Brampton—R. Lawrence, Felthorpe—H. le Strange, Hunstanton—B. B. Sapwell, Aylsham—C. B. Ticehurst, Lowestoft and J. H. Walter, Drayton.

*Essex*:—Miss K. M. Courtauld, Colne Engaine—Messrs H. S. Bell, Earls Colne—G. Brunwin, Great Bardfield and H. W. Page, Boxted.

*Cambridgeshire*:—Mr F. N. Webb, Babraham, and Mr A. J. Burgess, Gravel Hill Farm, Cambridge.

My thanks are also due to Mr Warburton, who has helped me considerably both in the identification of insects and also in the general management; to Mr Scott, curator in Entomology in the University of Cambridge, for help in identifying insects, and also for the use of a large collection of insects for reference; and to the Department of Agriculture, Cambridge, for defraying the expenses of the investigation.

To state briefly, the method of working was as follows:—The birds when killed were sent in with field notes, giving the particulars before mentioned, and on arrival they were cut open and examined. In both species of birds systematically investigated there was no crop, and consequently most of the material came from the gizzard and proventriculus, although a little was sometimes found in the oesophagus. Below the gizzard there was not much that could be identified, so that as a rule the intestines were not examined. The method of separating the stomach contents was essentially the same as that adopted by

Newstead (12). The materials were washed out into a white porcelain dish by a jet of water and then washed and roughly separated by a process of sedimentation; afterwards each separate article was picked out with forceps and placed in a watchglass. These pieces, after being identified and their numbers counted, were mounted on a sheet of cardboard.

The numbers of the insects given in the records below were arrived at by counting those parts which were best preserved. The materials were divided up, with reference to Agriculture, into three main divisions —injuries, benefits and neutral—and the two former subdivided into vegetable and animal matter, thus following roughly the classification of Gilmour (4) and more closely that adopted lately by Mason and Lefroy (11). It was often difficult to decide under which heading a certain insect should be placed, for in most cases the species was not determined. Many genera have been classed together owing to the difficulty in identifying them exactly from the remains; thus Carabid beetles are all classed together and placed in the "injurious" column, because a large number are carnivorous. In a few cases the genus is only approximate; for example, any Gastropod of the Helix type has been referred to as Helix. The majority of the insects and weed seeds were identified by comparing them with collection specimens, the grain by the structure of the husk and form of the starch grains, and the leaves by the appearance of the epidermis and position of the girders and ribs in transverse section.

## PART II. THE STARLING.

The starling appeared to be a suitable bird for investigation because a wide difference of opinion exists concerning its value to Agriculture. Slater (17) states that it is "normally one of the most useful birds we have"; while Archibald (1) has noted that it seems to be changing its habits for the worse, so far as the taking of grain is concerned; again, in a leaflet of the Board of Agriculture (20) the view is expressed that its usefulness far more than outweighs the occasional harm done. Of late there have been many complaints by farmers concerning the damage done by it, resulting in the recent deletion of the bird's name from the protected list in the county of Middlesex and the action against it by the West Somerset Farmers' Club.

Accounts of investigations concerning the food of the starling have been published by Gilmour (4), who, examining birds from one estate

in Fifeshire, came to the conclusion that they were beneficial; by Newstead (12), who investigated particularly the food of the young; by Kelso (10), who showed that seed wheat was taken; and by Collinge (3) who states that "the food during the first six months of the year was distinctly of an insectivorous character in the vicinity of the city of Birmingham."

## TABLE I.

This table shows the localities where the birds were killed each month. It may be objected, from what follows below that the localities were not represented proportionately to the number of birds frequenting those localities. To me it seems probable that the numbers are more or less approximate, as a natural result of the means by which they were obtained. In support of this, field notes concerning the places that the birds frequented were in general agreement with the numbers given in the table below. It should be pointed out here that no attempt was made to investigate the effect of the bird on fruit growing.

## TABLE I. STARLINGS.

*Number and localities of birds examined each month.*

| Locality | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meadow | 4 | 8 | 7 | 9 | 7 | 8 | 0 | 4 | 0 | 0 | 0 | 6 | 53 |
| Marsh | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 6 |
| New ley | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| Hay field | 0 | 0 | 0 | 0 | 5 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 7 |
| With cattle | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 12 |
| Sheep fold | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 9 |
| Following plough | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 5 |
| Ploughed land | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 10 |
| Turnips | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 | 2 | 0 | 0 | 8 |
| Garden | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Farmyard | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Seed Barley | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Spring Wheat | 1 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| Autumn Wheat | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 12 | 2 | 33 |
| Stubble | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 1 | 0 | 0 | 7 |
| Feeding young | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Tree or flying | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 5 | 1 | 3 | 5 | 20 |
| Nestlings | 0 | 0 | 0 | 12 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| Unknown | 0 | 6 | 0 | 2 | 3 | 0 | 2 | 4 | 0 | 2 | 0 | 0 | 19 |
| Totals | 17 | 24 | 29 | 29 | 23 | 16 | 11 | 13 | 15 | 25 | 19 | 18 | 239 |

TABLE II.  *Extracts from the record.*

| No. | Date | Time | Place | Locality | Weather | Sex | Injuries | | Benefits | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Crops | Insects, etc. | Weeds | Insects, etc. | Neutral |
| 9 | Feb. 6th | noon | Brampton, Norfolk | Following plough | Fine | ♂ | A piece of oat husk | 32 Centipedes<br>4 Carabid beetles<br>1 Staphylinid beetle larva | — | 1 Millipede<br>2 Earwigs<br>33 Anthomyid (?) fly pupae<br>7 Noctuae larvae and 3 pupae<br>29 Wireworms<br>1 Click beetle | 1 Spider<br>2 Dipterous larvae |
| 95 | March 29th | 12.30 p.m. | Felthorpe, Norfolk | Meadow | Mild | ♂ | — | 1 Centipede | — | 75 Leather-jackets<br>1 Click beetle<br>3 Weevils (Sitones)<br>3 Gastropods (Helix) | 4 Dipterous larvae<br>2 pieces of grass |
| 247 | July 5th | 5.30 p.m. | Brampton, Norfolk | Meadow with cattle | Warm | Young | — | 3 Carabid beetles | — | 18 Heteroptera<br>2 Ants<br>1 Weevil (Otiorhynchus)<br>4 Halticid beetles | 2 Gnats |
| 396 | Dec. 12th | 12.30 p.m. | Framingham Pigot, Norfolk | Wheat | Fine | ♀ | 8 grains of wheat and much husk | 4 Centipedes<br>2 Earthworm eggs<br>1 Carabid beetle<br>1 Staphylinid beetle | — | 3 Weevils<br>1 Gastropod (Helix) | 6 Spiders<br>1 Muscid fly<br>2 Dipterous pupae<br>60 Dipterous larvae |

TABLE III. *December* (19 *Starlings*).

| | Injuries | | | Benefits | | | Neutral | | |
|---|---|---|---|---|---|---|---|---|---|
| | Article of diet | Times occurred | Number occurred | Article of diet | Times occurred | Number occurred | Article of diet | Times occurred | Number occurred |
| *Seeds* | Wheat | 10 | 87 | Polygonum aviculare | 2 | 2 | — | | |
| | Barley | 1 | 1 | Polygonum Convolvulus | 1 | 1 | | | |
| | | | | Unidentified | 1 | 1 | | | |
| *Vegetation, etc.* | Wheat husk | 17 | much | Nettle leaf | 3 | moderate | Grass and unidentified leaf | 8 | little |
| | Barley husk | 1 | little | | | | Moss | 2 | little |
| | Cotton-seed husk | 2 | little | | | | | | |
| *Insects, etc.* | Earthworm eggs | 1 | 2 | Leather-jackets | 2 | 6 | Spiders | 14 | 68 |
| | Centipedes | 5 | 10 | Lepidopterous larvae | 9 | 29 | Dipterous pupae | 6 | 19 |
| | Staphylinid beetle larvae | 7 | 10 | Noctua | 6 | 25 | Dipterous larvae | 3 | 63 |
| | Staphylinid beetles | 12 | 29 | Various | 3 | 4 | Muscid flies | 1 | 1 |
| | Carabid beetle larvae | 3 | 4 | Wireworms | 1 | 8 | Scarabid beetles | 1 | 3 |
| | Carabid beetles | 14 | 94 | Click beetles | 5 | 9 | Unidentified | 2 | few |
| | Coccinellid beetles | 1 | 1 | Haltieid beetles | 1 | 1 | | | |
| | | | | Weevils | 17 | 280 | | | |
| | | | | Sitones | 16 | 254 | | | |
| | | | | Otiorhynchus | 5 | 7 | | | |
| | | | | Apion | 2 | 4 | | | |
| | | | | Various | 5 | 15 | | | |
| | | | | Gastropods | 16 | 240 | | | |
| | | | | Helix | 14 | 58 | | | |
| | | | | Stenogyridae (?) | 1 | 1 | | | |
| | | | | Pupidae (?) | 1 | 11 | | | |
| | | | | Various | 1 | 170 | | | |
| *Miscellaneous* | — | | | — | | | Tea leaves | 1 | little |

## TABLE II.

This table shows the way in which the record of the details concerning each bird was kept. The first five columns consist of field notes; while the rest, with the exception of column six, comprise the results of investigating the stomach contents.

## TABLE III.

This table is an example of those which have been compiled in order to analyse the mass of details contained in the record, of which extracts have been given in Table II. The object in view is to present at a glance the food of the bird for any particular month, so that some idea can be formed of its benefits or injuries at that time of year. Such tables and numbers are necessary, for as has been pointed out by Theobald (18), if we rely only on our own judgment, a certain amount of prejudice must come in. The actual figures for each month have not been given, as they are embodied in Table IV, but the most important features are included in the discussion of the monthly results below.

The following is a description of the food taken during each month :—

*February.*—The bulk of the food consisted of insects, etc., of which the most important were Centipedes, Leather-jackets, Wireworms, Gastropods and Bibio fly larvae. Some grain with a considerable amount of husk was also taken; much of this was undoubtedly from dung, as the general appearance and presence of Mycetophilid fly larvae indicated. Birds which came from sheepfolds, in addition to the common insects, contained pieces of oat grain, cotton seed husk and cut hay, which were probably taken from the feeding troughs. No. 9 [Table II] is an example of the large number of insects, etc., taken when the bird is following the plough.

*March.*—The food consisted chiefly of insects, etc., of which Staphylinid beetles and their larvae, Carabid beetles, Click beetles, Weevils, Gastropods, Spiders and Mycetophilid fly larvae were present in greatest numbers. The corn husk taken was almost entirely from dung. Weed seeds occurred very occasionally. Tea leaves with odds and ends of rubbish found in several birds indicate their habit of searching in dust heaps. The large number of insects consumed by birds frequenting meadow land will be seen in No. 95 [Table II].

*April.*—A short spell of cold weather at the beginning of the month

coincided with the period at which some of the spring sown corn was coming up and as a result much grain and husk (both barley and wheat) was taken. At the same time, however, many insects etc. were eaten, the majority being Staphylinid and Carabid beetles, Leather-jackets, Ants, Wireworms, Click beetles, Weevils and Gastropods. On two occasions during the frosty weather pieces of swede bulb were taken.

*May.*—The food consisted almost entirely of insects, many of which however were beneficial. The food of the young (which is included in this month's results) consisted for the most part of Carabid beetles, Leather-jackets, Click beetles and Weevils.

*June.*—The bulk of the food consisted of insects, of which the most important were Carabid beetles, Leather-jackets, Ants and their pupae, Click beetles and Weevils. A little grain [Hemp, Canary grass and Oats] occurred; this was probably stolen from pheasants food although presumably there was a plentiful supply of insects.

*July.*—The food again consisted mostly of insects. Grain [hemp etc.] was taken in small quantities, presumably from pheasants food. Evidence concerning the effect of the bird on fruit growing occurred during this month, strawberries being found on three occasions. It was quite a common sight at this time of the year to see flocks of starlings assembling round cattle on the meadows and clover leys; these birds on being examined were found to contain a large number of Heteroptera, Weevils and other insects (No. 247, Table II). It is probable that starlings assemble in these localities for the insects which are disturbed by the treading of the cattle, rather than for the reputed ticks.

*August.*—Insects, chiefly Carabid beetles, Lepidopterous larvae, Weevils and Dipterous pupae, form the bulk of the diet. Strawberries occurred twice, while towards the end of the month a few elderberries were taken.

*September.*—The food this month consisted mainly of insects etc. and berries. Among the insects etc., Carabid beetles, Earwigs, Lepidopterous larvae, Weevils and Spiders were those which occurred in largest numbers. The most noteworthy point this month was the attack on elderberries; many of the stomachs and their contents were stained a deep purple. Blackberries were also taken in small quantities. In one case corn (wheat) was taken from the stubbles.

*October.*—Of the insects which formed the greater portion of the diet Staphylinid beetles, Lepidopterous larvae, Weevils, Dipterous larvae and pupae and Scarabid beetles were the most conspicuous.

In the beginning of the month elderberries and blackberries were taken and towards the end some corn husk was found.

*November.*—This month was marked by the great amount of seed wheat and husk found; 92 % of the birds examined contained this material.

The damage is done just as the blade is appearing through the ground. A hole is dibbled round the blade and the germinating grain pulled out and eaten; as often as not the blade is broken off at the same time, this depending on the age of the plant. On examining the stomach contents of these birds one finds sound grain, that which has just germinated, the empty husks, and usually embryos (scutellum) often with the roots and occasionally the soft part of the blade attached. On no occasion was the foliage of wheat found. At the same time, however, a number of insects etc. were eaten, chiefly Staphylinid beetles and their larvae, Lepidopterous larvae, Weevils, Gastropods, Spiders and Dipterous larvae. In a bird from Hunstanton the remains of a crab were found.

*December.*—Much seed corn (wheat) and husk were again taken during this month, 89 % of the birds containing this material (No. 396, Table II). It was found that the later sown wheat was damaged most; when it grows beyond a certain stage (probably when all the endosperm has been used up) the birds do not interfere with it. Many insects etc. were also taken; the most important of these were Carabid beetles, Weevils, Gastropods, Spiders and Dipterous larvae (Table III).

*January.*—A smaller amount of corn was taken during this month and some of the husk was probably from dung. Insects etc. occurred in large numbers; the most important of these were Staphylinid and Carabid beetles, Ants, Click beetles, Weevils, Gastropods, Spiders and Mycetophilid, Stratiomyid and other Dipterous larvae. An unusual number of weed seeds were present; this was probably due to the frosty weather, which seems to cause a very heterogeneous diet. The occurrence of woodlice is interesting in connection with the observations of Newstead (12) who states that these were picked up but rejected by starlings. It appears from the present investigation that they are only eaten under stress of weather.

## TABLE IV.

This table represents the subject from the point of view of the food article. It shows the time of year when the various food substances

were eaten and also the quantities in which they were taken. The table is a modified form of that used by Hollrung (6) for rooks. The monthly results, of which Table III is an example, have been calculated down to a common basis of ten stomachs for the purpose of comparison. The numbers that occurred and not the number of occurrences have been used wherever possible in the calculations, for it was considered that the latter would present a wrong idea of the position occupied by the substance in the bird's diet.

To mention briefly a few of the facts shown by this table :—

Wheat is taken to a large extent in the autumn and in smaller quantities in the spring. Barley and oats occur mainly in the spring.

There is no regularity in the occurrence of weed seeds. Of husk, wheat is the most important and occurs most frequently during the winter and spring months. With regard to insects etc., Centipedes, Staphylinid and Carabid beetle larvae, Millipedes, Wireworms and Click beetles occur frequently during the autumn, winter and spring months; but practically none are taken during the summer. This might be accounted for by the fact that ploughing goes on principally during the former period. As the occurrence of many insects is normally limited to certain times of the year (Telephorid beetles, Heteroptera, Bibio flies, etc.), it is only natural that they should be taken by starlings during the periods at which they are most plentiful. The number of Lepidopterous larvae taken rises in August, September and October; it is possible that this may be due to their exposure as a result of the corn harvest. With Weevils, there are periodic rises and falls in the numbers taken ; this may possibly in some measure be due to the occurrence of successive broods. Gastropods are taken in larger numbers during the winter months; this perhaps is due to the lack of cover at this time of year. The consumption of spiders appears fairly constant throughout the year, although on the whole tending to rise during the winter months. The occurrence of Mycetophilid fly larvae is almost entirely limited to the winter ; Stratiomyid and Dipterous larvae follow almost the same course, while Dipterous pupae and Scarabid beetles are taken mostly in the autumn. The miscellaneous substances occur haphazard although perhaps slightly more often during the winter months. With regard to the material classed as grass and unidentified leaf, it is thought that its occurrence is accidental in nature, as it was generally found in very small quantities and was often tangled as though it had been wrapped round a Leather-jacket or picked up with some other object.

TABLE IV. STARLINGS.

| Month:— | February | March | April | May | June | July | August | September | October | November | December | January | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weather:— | Fine-cold | Fine-warm | Snow then warm | Warm and dry | Dry then showery | Hot and dry | Warm and dry | Hot and dry then showers | Damp | Fine with wet spells | Wet and warm | Wet then frost and snow | |
| No. of stomachs from which calculated | 17 | 24 | 29 | 29 | 23 | 16 | 11 | 13 | 15 | 25 | 19 | 18 | 23·0 |
| **SEEDS:—** | | | | | | | | | | | | | |
| *(a) Crop:—* | | | | | | | | | | | | | |
| Wheat | 2·9 | 0 | 36·2 | 0 | 0 | 3·1 | 0 | 15·4 | 0 | 72·0 | 45·8 | 8·9 | 15·4 |
| Barley | 17·0 | 0 | 5·9 | 0 | 0 | 0 | 0 | 0 | ·7 | 0 | ·5 | 1·7 | 2·1 |
| Oats | 4·7 | 2·1 | 3·1 | 0 | ·4 | ·6 | 0 | 0 | ·7 | 0 | * | 1·7 | 1·1 |
| Beans | 0 | 0 | ·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·02 |
| Maize | 0 | 0 | 0 | 0 | 0 | 1·9 | 0 | ·8 | 0 | 0 | 0 | 0 | ·1 |
| Rye-grass | 0 | 0 | ·3 | 0 | 0 | 1·2 | 0 | 0 | 0 | 0 | 0 | 1·7 | ·3 |
| Trefoil | 1·2 | 0 | 0 | 0 | 4·8 | 33·1 | 0 | 0 | 0 | 0 | 0 | 0 | 3·1 |
| Hemp | 0 | 0 | 0 | 0 | 1·3 | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Canary-grass | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| *(b) Weeds:—* | | | | | | | | | | | | | |
| Papaver Rhoeas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12·8 | 1·1 |
| Stellaria media (?) | 0 | ·4 | ·7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 |
| Lychnis | 0 | 3·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·03 |
| Vetch | 0 | ·1 | ·7 | 0 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·3 |
| Plantago lanceolata | 0 | 2·1 | ·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Polygonum aviculare | ·6 | 0 | 0 | 0 | ·4 | 0 | 0 | 0 | 0 | 2·0 | 1·0 | 2·2 | ·6 |
| Polygonum Convolvulus | 1·2 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·6 | ·5 | 1·1 | ·3 |
| Polygonum lapathifolium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·8 | 0 | 0 | 0 | 0 | ·03 |
| Rumex crispus | ·6 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3·3 | ·7 |
| Rumex Acetosella | ·6 | 0 | 0 | 0 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | ·5 | ·1 |
| Chenopodium album | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Galium Aparine | 0 | 0 | 0 | 0 | ·4 | 0 | 0 | ·8 | 0 | 0 | 0 | 0 | ·03 |
| Poa annua | 0 | 0 | 0 | 4·4 | 17·8 | 0 | 0 | 0 | 3·3 | ·4 | ·5 | 2·2 | 1·8 |
| Unidentified | 0 | 0 | 0 | 0 | ·9 | 0 | 0 | 1·5 | 0 | 0 | 0 | 0 | ·7 |
| *(c) Neutral:—* | | | | | | | | | | | | | |
| Elderberry | 0 | 0 | 0 | 0 | 0 | 0 | 90·9 | 855·4 | 13·3 | 0 | 0 | 0 | 79·9 |
| Blackberry | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33·1 | 44·7 | 2·8 | 0 | 0 | 6·7 |
| **VEGETATION, ETC.:—** | | | | | | | | | | | | | |
| *(a) Crop:—* | | | | | | | | | | | | | |
| Wheat husk | 1·8 occ. | 2·5 occ. | 3·4 occ. | 0 | ·4 occ. | 0 | 0 | ·8 occ. | ·7 occ. | 8·4 occ. | 8·9 occ. | 4·4 occ. | 2·6 occ. |
| Barley husk | 1·8 occ. | ·4 occ. | 2·7 occ. | 0 | ·4 occ. | 0 | ·9 occ. | 0 | 4·0 occ. | ·4 occ. | ·5 occ. | 2·2 occ. | 1·1 occ. |
| Oat husk | 4·7 occ. | ·8 occ. | ·7 occ. | ·7 occ. | 1·7 occ. | 2·5 occ. | ·9 occ. | 1·5 occ. | 1·3 occ. | ·4 occ. | 0 | 2·8 occ. | 1·5 occ. |
| Pea or Bean husk | 0 | 0 | 0 | 0 | ·4 occ. | 0 | ·9 occ. | 0 | ·7 occ. | 0 | 0 | 0 | ·16 occ. |
| Cotton-seed husk | 1·8 occ. | 0 | ·3 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·1 occ. | 1·1 occ. | ·3 occ. |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hay and Straw | 2·4 occ. | 1·2 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·1 occ. | ·4 occ. |
| Clover pods | 0 | 25·0 | ·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2·1 |
| Swede (bulb) | ·6 occ. | 0 | ·7 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 occ. |
| Potato | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·05 occ. |
| Pear skin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·05 occ. |
| Apple skin | 0 | 0 | 0 | 0 | 0 | ·6 occ. | 0 | 0 | 0 | 1·2 occ. | 0 | 0 | ·1 occ. |
| Strawberries | 0 | 0 | 0 | 0 | 0 | 1·9 occ. | 1·8 occ. | 0 | 0 | 0 | 0 | 0 | ·3 occ. |
| **(b) Weeds:—** | | | | | | | | | | | | | |
| Compositae heads | ·6 | ·8 | 2·4 | 0 | 1·7 | ·6 | 3·6 | 0 | 0 | 0 | 0 | 2·8 | 1·1 |
| Nettle leaf | ·6 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·6 occ. | 2·8 occ. | ·4 occ. |
| **(c) Neutral:—** | | | | | | | | | | | | | |
| Fibrous roots | 2·4 occ. | ·8 occ. | ·7 occ. | ·7 occ. | ·4 occ. | 0 | 2·7 occ. | 0 | 1·3 occ. | ·8 occ. | 4·2 occ. | 3·1 occ. | ·5 occ. |
| Leaves (unidentified) | 5·3 occ. | 5·8 occ. | 5·5 occ. | 6·2 occ. | 6·1 occ. | 3·1 occ. | 0 | 0 | 0 | 3·2 occ. | 0 | 3·9 occ. | 4·0 occ. |
| **INSECTS, ETC.:—** | | | | | | | | | | | | | |
| **(a) Beneficial:—** | | | | | | | | | | | | | |
| Earthworms' eggs | 7·6 | 10·0 | 5·2 | 8·9 | 0 | 0 | 0 | 0 | 0 | 1·6 | 1·0 | 1·1 | 2·2 |
| Earthworms | ·6 | 2·5 | ·7 | 0 | 0 | ·6 | ·9 | 0 | 2·0 | ·8 | 0 | 0 | ·8 |
| Centipedes | 24·1 | 7·1 | 5·9 | 4·5 | ·9 | ·6 | ·9 | ·8 | 8·7 | 3·2 | 5·3 | 6·7 | 5·6 |
| Syrphid flies | 0 | 0 | ·3 | 0 | ·4 | 0 | 0 | 0 | 1·3 | 0 | 0 | 0 | ·2 |
| Therava fly larvae (?) | 0 | 0 | 1·7 | 0 | 0 | 6·9 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Ichneumon flies | 0 | 0 | 0 | 0 | 10·4 | 10·0 | 0 | 0 | 1·3 | 0 | 0 | 0 | 1·5 |
| Staphylinid beetles | 3·6 | 43·7 | 19·3 | 16·9 | 6·9 | 3·7 | 5·5 | 7·7 | 22·0 | 22·8 | 15·3 | 28·9 | 16·9 |
| Staphylinid beetle larvae | 9·4 | 16·7 | 9·0 | 1·7 | 1·7 | 35·1 | 26·4 | 27·7 | 3·3 | 20·0 | 5·3 | 10·5 | 6·8 |
| Carabid beetles | 11·8 | 28·3 | 16·9 | 40·0 | 35·2 | 0 | 0 | 0 | 14·7 | 7·6 | 49·5 | 26·7 | 26·7 |
| Carabid beetle larvae | 4·1 | 6·7 | 1·7 | 0 | 0 | 0 | 0 | ·8 | 0 | ·8 | 2·1 | 13·3 | 2·4 |
| Dytiscid beetles | 0 | 0 | 1·0 | 0 | 0 | 1·9 | 0 | 0 | 0 | 0 | 0 | 0 | ·08 |
| Dytiscid beetle larvae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Telephorid beetles | 0 | 0 | 0 | 3·8 | 3·0 | 1·2 | 0 | 0 | 0 | ·8 | 0 | ·5 | ·6 |
| Coccinellid beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5·3 | 3·6 | ·5 | 0 | ·9 |
| **(b) Harmful:—** | | | | | | | | | | | | | |
| Millipedes | 0·6 | 12·9 | 9·3 | 2·1 | 6·5 | 1·9 | 1·9 | ·8 | 6·0 | 1·2 | 0 | 1·1 | 4·1 |
| Heteroptera | 0 | 0 | 0 | ·7 | 6·1 | 21·3 | 9·1 | 12·3 | 3·3 | 2·8 | 0 | 0 | 4·6 |
| Aphids | 0 | 0 | 0 | 0 | 0 | ·6 | 2·7 | 30·0 | 0 | 0 | 0 | 6·7 | ·05 |
| Earwigs | 2·4 | 2·5 | 1·4 | 1·7 | 4·3 | 7·5 | 0 | 0 | 4·0 | 3·6 | 0 | 0 | 5·7 |
| Hessian fly pupae (?) | 2·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Leather-jackets | 30·0 | 40·4 | 15·9 | 35·5 | 15·6 | 0 | 0 | 0 | 0 | ·4 | 3·2 | 2·9 | 11·9 |
| Crane flies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4·0 | 0 | 0 | ·03 |
| Anthomyid fly pupae (?) | 19·4 | 0 | ·7 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 | 0 | 2·0 | 2·0 |
| Bibio fly larvae | 44·1 | 2·1 | 0 | 11·0 | 0 | 0 | 0 | 0 | 0 | 4·0 | 0 | 2·2 | 4·0 |
| Bibio flies | 0 | 0 | 0 | 0 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·9 |
| Lepidopterous larvae — Noctua | 8·8 | 3·3 | 3·1 | 2·1 | {11·3 | {7·5 | 47·3 | 26·1 | 19·3 | ·8 | 13·2 | 12·8 | 8·1 |
| Lepidopterous larvae — Various | 1·8 | ·8 | ·7 | ·7 | | | 2·7 | 9·2 | 4·7 | 16·8 | 2·1 | 8·9 | 8·9 |
| *(Lepidopterous larvae total)* | 10·6 | 4·1 | 3·8 | 2·8 | 11·3 | 7·5 | 50·0 | 35·3 | 24·0 | 17·6 | 15·3 | 21·7 | 17·0 |
| Lepidopterous pupae | 1·8 | 0 | 0 | 0 | ·4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**TABLE IV.** (*continued*).

| Month:— | February | March | April | May | June | July | August | September | October | November | December | January | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weather:— | Fine-cold | Fine-warm | Snow then warm | Warm and dry | Dry then showery | Hot and dry | Warm and dry | Hot and dry then showers | Damp | Fine with wet spells | Wet and warm | Wet then frost and snow | |
| No. of stomachs from which calculated | 17 | 24 | 29 | 29 | 23 | 16 | 11 | 13 | 15 | 25 | 19 | 18 | 239 |
| INSECTS, ETC.:— | | | | | | | | | | | | | |
| *Harmful* (cont.):— | | | | | | | | | | | | | |
| Cephid sawflies | 0 | 0 | 0 | 0 | 0 | 6·9 | 2·7 | 0 | 0 | 0 | 0 | 0 | ·8 |
| Sawfly pupae (?) | 0 | 0 | 0 | 0 | 8·7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·7 |
| Ants | 0 | 10·8 | 22·4 | 2·1 | 19·1 | 22·5 | 1·8 | 20·8 | 10·7 | 0 | 0 | 33·9 | 12·0 |
| Ant pupae | 0 | 0 | 0 | 0 | 129·1 | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | 10·8 |
| Wireworms | 21·8 | 1·7 | 46·2 | 6·2 | 5·2 | 0 | 0 | 0 | 8·0 | 1·2 | 4·2 | 16·7 | 9·3 |
| Click beetles | 4·1 | 20·4 | 31·3 | 30·3 | 13·9 | 2·5 | 0 | 0 | 0 | 1·6 | 4·7 | 27·8 | 11·4 |
| Halticid beetles | ·6 | 0 | ·3 | 0 | 0 | 5·0 | 1·8 | ·8 | 8·0 | ·4 | ·5 | 0 | 1·4 |
| Chrysomelid beetles | 0 | 0 | 0 | ·3 | 0 | 6·3 | 0 | ·8 | 8·0 | ·4 | 0 | 0 | ·6 |
| Anobium | 0 | 0 | 0 | 0 | 0 | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | ·05 |
| Weevils | | | | | | | | | | | | | |
| Sitones | 14·7 | 34·6 | 22·7 | 6·2 | 8·6 | 29·4 | 86·4 | 49·2 | 18·7 | 89·2 | 113·7 | 92·8 | 56·9 |
| Otiorhynchus | 1·2 | 8·7 | 10·0 | 6·9 | 5·2 | 3·7 | 3·6 | 8·5 | 3·3 | 7·6 | 3·8 | 5·5 | |
| Apion | 0 | 1·2 | 0 | ·7 | ·4 | 0 | 0 | 0 | 0 | 0 | 2·1 | ·5 | |
| Various | 0 | 2·5 | 1·4 | 1·0 | 4·3 | 12·5 | 3·6 | 1·5 | 0 | ·8 | 7·9 | 8·3 | |
| | 15·9 | 47·0 | 34·1 | 14·8 | 18·5 | 45·6 | 93·6 | 59·2 | 22·0 | 97·6 | 127·5 | 107·1 | 56·9 |
| Gastropods | | | | | | | | | | | | | |
| Helix | 52·9 | 82·5 | 61·4 | ·5·2 | 7·8 | 20·0 | 11·8 | 14·6 | 3·3 | 16·0 | 30·5 | 113·9 | |
| Pupidae (?) | ·6 | 3·3 | ·7 | ·3 | 1·7 | 0 | 0 | ·8 | 1·3 | 4·8 | 5·8 | 2·2 | |
| Stenogyridae (?) | 2·3 | 9·6 | 5·2 | 1·7 | 0 | 0 | 0 | ·3 | ·7 | ·4 | ·5 | 12·8 | |
| Limnaea | 1·8 | 2·1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·5 | |
| Slugs | 2·9 | 2·5 | 0 | ·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Snails' eggs (?) | 1·2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Various | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89·5 | 0 | |
| | 61·7 | 100·0 | 67·3 | 7·5 | 9·5 | 20·0 | 11·8 | 16·2 | 5·3 | 21·2 | 126·3 | 129·4 | 48·0 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **(c) Neutral:—** | | | | | | | | | | | | | |
| Crabs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 | 0 | 0 | ·03 |
| Wood lice | 0 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2·8 | ·3 |
| Spiders | 15·9 | 59·6 | 14·1 | 5·9 | 2·6 | 22·5 | 22·7 | 30·8 | 2·7 | 25·6 | 35·8 | 43·9 | 23·5 |
| Spiders' eggs (?) | 0 | 0 | 0 | 0 | many | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — |
| Harvest Spiders (Phalangideae) | ·6 | ·4 | 0 | 0 | 0 | 20·0 | 3·6 | 0 | 2·0 | 0 | 0 | 0 | 2·1 |
| Collembola | 9·4 | 11·2 | 0 | 0 | 0 | 1·2 | 0 | ·8 | 0 | 0 | 0 | 0 | ·2 |
| Stratiomyid fly larvae | 130·0 | 102·5 | 6·2 | 3·1 | ·4 | 0 | 10·9 | 0 | 0 | ·4 | 0 | 56·7 | 8·3 |
| Mycetophilid fly larvae | 6·5 | 5·4 | 38·6 | 1·7 | 0 | 3·1 | 14·5 | ·8 | 44·0 | 136·4 | 33·1 | 64·4 | 27·9 |
| Dipterous larvae | 4·1 | 4·2 | 3·8 | ·3 | 0 | 40·0 | 60·0 | 1·5 | 108·7 | 12·4 | 7·9 | 268·9 | 43·2 |
| Dipterous pupae | ·6 | 2·9 | 3·1 | ·3 | ·9 | 4·4 | ·9 | ·8 | 4·0 | 0 | ·5 | 11·1 | 21·2 |
| Muscid flies | 0 | 0 | 0 | 0 | ·9 | 3·1 | 0 | ·8 | 0 | 1·6 | 0 | ·5 | 1·3 |
| Gnats | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·5 | ·5 |
| Choropid flies | 1·2 | 3·3 | 6·5 | ·3 | ·9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·01 |
| Scarabid beetles | ·6 | 1·2 | 3·8 | 2·1 | ·9 | ·6 | 1·8 | 1·5 | 63·3 | 4·4 | 1·6 | 2·8 | 7·3 |
| Parnid beetles | 0 | ·8 | 1·4 | 1·0 | 0 | ·6 | 0 | 0 | 1·3 | 0 | 0 | 0 | ·6 |
| Hydrophilid beetles | 0 | ·4 | 0 | ·7 | 0 | 0 | 0 | 0 | 3·3 | 0 | 0 | 0 | ·4 |
| Silphid beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 |
| Unidentified | 1·2 occ. | ·8 occ. | ·3 occ. | ·3 occ. | ·4 occ. | 1·2 occ. | 0 | 0 | 0 | 1·6 occ. | 1·0 occ. | 2·8 occ. | ·8 occ. |
| **MISCELLANEOUS:—** | | | | | | | | | | | | | |
| Bone (and flesh) | 1·2 occ. | 1·2 occ. | 0 | 0 | 1·3 occ. | ·6 occ. | 0 | ·8 occ. | 0 | ·4 occ. | 0 | 0 | ·5 occ. |
| Egg-shell | 0 | ·4 occ. | 0 | 0 | ·4 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·07 occ. |
| Shell (pink) | 0 | ·8 occ. | 0 | 0 | 0 | 0 | 0 | ·8 occ. | 1·3 occ. | 0 | 0 | 0 | ·1 occ. |
| Coal | 0 | ·4 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·5 occ. | 0 | ·2 occ. |
| Orange peel | 0 | ·4 occ. | 0 | 0 | 0 | 0 | 0 | 0 | ·7 occ. | ·4 occ. | 0 | 0 | ·03 occ. |
| Tea leaf | 0 | ·8 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 occ. | 0 | 0 | ·2 occ. |
| Wood | ·6 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·7 occ. | ·8 occ. | 0 | 0 | ·1 occ. |
| Bacon rind | ·6 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 occ. | 0 | 0 | ·1 occ. |

In order to obtain a total estimate of the bird's food, the monthly results have been averaged and placed in a column at the end of Table IV; thus these figures represent the numbers of each article which would be found in ten average stomachs. These numbers may be balanced against one another on some such assumption that 10 grains of wheat = 1 wireworm, as has been done by Hollrung (6) in the case of the rook. Looked at in this way it seems to be a beneficial bird on the whole; but we have yet to consider the question of migration. It is stated by Saunders (15) that the starling migrates to this country from Northern Europe in the autumn (our birds going further south and west) and returns in the spring. One definite case was noted in the course of this investigation; a bird was killed near Thetford, Norfolk, which had been ringed a few days before in Viborg, Denmark, by C. Mortensen who is investigating migration. It is the autumn migrants from abroad which do the damage to seed corn, but at the same time it ought to be pointed out that they are eating considerable numbers of insects, etc. To abate this plague there seem to be two courses open (since the birds are very difficult to scare off the fields), either (i) to dress the seed corn with something that will render it distasteful to birds, or (ii) as an extreme measure if (i) fails, to kill off the autumn migrants in large numbers.

With reference to (i) experiments undertaken by the German Imperial Biological Institute (22) have shown that corn dressed with Prussian blue, creolin or powdered aloes was rendered distasteful to rooks. Numerous tar preparations have been put forward, but they seem open to the objection that they retard germination, although a dressing mentioned by Sawyer (16) is supposed not to have this effect.

With regard to (ii) it is a matter of general belief and has been recorded by Saunders (15), Hooper (7) and Collinge (3) that starlings have increased enormously in numbers of late years. It has been suggested that this is due to a series of mild winters, but it may possibly be a result of their preservation in those places from which they migrate (21).

It is probable that this increase in numbers has been out of proportion to that of their insect food, so that wheat has been taken in order to eke out the diet. It should then follow that if their numbers were reduced, insect food would be in sufficient abundance to form the entire diet without the addition of grain. Perhaps the easiest way to reduce their numbers would be by the use of poison; this course seems open to the objection that the poison might be misused, but this ought not to occur if under proper control.

CONCLUSIONS.

(1)    The starling is very beneficial during the late spring, summer and early autumn months, eating many harmful insects although a number of beneficial ones are also destroyed.

(2)    During the autumn, and to a less extent in the spring, much harm is done by the consumption of seed corn (particularly wheat); many harmful insects however are also destroyed during this period.

(3)    Owing to the fact of the bird's autumn and spring migrations, the remedies suggested are, either (i) to dress the seed corn with something which renders it distasteful to birds; or (ii), if suggestion (i) cannot be carried out successfully, to kill off the autumn migrants in large numbers.

PART III.    THE LARK.

This bird was considered a suitable subject for investigation, as, on the one hand, most of the standard books on birds regard it as beneficial to agriculture; whereas, on the other hand, Hooper (7) states that it is destructive to wheat, vetches, cabbages, etc., and farmers have complained of the damage to clover leys and seed wheat. The only records of its stomach contents that could be found were those published by Newstead (12).

TABLE I.    LARKS.

*Number and localities of birds examined each month.*

| Locality | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| New ley ................. | 10 | 7 | 4 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 10 | 46 |
| Lucerne, etc. ........... | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Meadow ................. | 0 | 4 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 7 |
| Hay field................. | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| Ploughed fields ......... | 1 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 9 |
| Wheat ................. | 2 | 7 | 7 | 1 | 0 | 0 | 0 | 0 | 1 | 8 | 5 | 2 | 33 |
| Spring sown corn ...... | 1 | 4 | 3 | 8 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| Stubbles ................. | 2 | 0 | 0 | 0 | 0 | 0 | 7 | 2 | 2 | 1 | 0 | 0 | 14 |
| Turnips ................. | 0 | 0 | 0 | 1 | 0 | 4 | 0 | 4 | 1 | 3 | 0 | 2 | 15 |
| Mangolds .............. | 0 | 0 | 0 | 0 | 6 | 4 | 0 | 0 | 3 | 0 | 0 | 0 | .13 |
| Railway cutting ...... | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Old stack bottom ...... | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Nests ................. | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Unknown .............. | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 3 |
| Totals................ | 18 | 27 | 16 | 20 | 13 | 13 | 10 | 6 | 7 | 13 | 13 | 15 | 171 |

TABLE II.  *Extracts from the record.*

| No. | Date | Time | Place | Locality | Weather | Sex | Injuries | | Benefits | | | Neutral |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Crops | Insects, etc. | Weeds | Insects, etc. | | |
| 19 | Feb. 10th | 10.30 a.m. | Drayton, Norfolk | Clover ley | Slight frost | ♂ | 9 pieces of clover leaf | — | 48 seeds of Polygonum aviculare 7 seeds of Rumex Acetosella | — | | — |
| 30 | Feb. 22nd | 3 p.m. | Boxted, Essex | Newly drilled land | Windy | ♂ | 2 grains of oats and many pieces of epidermis | — | 28 seeds of Polygonum aviculare 1 seed of Polygonum lapathifolium | — | | — |
| 162 | May 10th | 6 p.m. | Brampton, Norfolk | Barley with seeds | Warm, dry | ♂ | 44 seeds of Lolium and much husk | — | 1 seed of Poa annua | 1 Click beetle | | 2 pieces of leaf (unidentified) |
| 211 | June 1st | 1 p.m. | Framingham Pigot, Norfolk | Nest in wheat field | Sunny | Nestling | 2 pieces of oat husk | 1 Carabid beetle | — | 1 Noctua larva 3 Click beetles 1 Weevil (Sitones) | | 1 Spider |
| 314 | Sept. 21st | 1 p.m. | Framingham Pigot, Norfolk | Swedes | Sunny | — | — | — | 4 seeds of Polygonum Convolvulus 1 seed of Polygonum aviculare 1 seed of Rumex Acetosella 1 seed (unidentified) | 50 Aphids 1 Halticid beetle (H. nemorum) | | 1 Dipterous larva |

TABLE I.

This table shows the localities from which the birds were taken.

The small numbers examined in September and October indicate the great difficulty in obtaining birds during these months. This was probably due to a period of time elapsing between the dates at which our birds start southward and the foreign migrants arrive.

TABLE II.

This table consists of a few extracts from the record of the details concerning each bird. The arrangement is similar to that of the corresponding table for starlings.

TABLE III.

This table is an example of those which were made to show the monthly food. It was compiled in the same way as in the case of the starling. The results for each month are not given as they are embodied in Table IV. A discussion of the monthly results follows below.

The following is a description of the food taken each month :—

*February.*—During this month 78 % of the birds examined contained the leaves of some crop. The green parts of the leaf were eaten, and mostly, although not always, the smaller leaves were found; as far as could be seen the young shoots and stems were not taken (No. 19, Table II). Weed seeds formed the bulk of the diet and an insect was rarely taken.

*March.*—Weed seeds, of which Polygonum aviculare is by far the most important, formed the bulk of the diet. Corn in small quantities was taken from the spring sowings (No. 30, Table II), and, as none had germinated, it was concluded that it was taken soon after sowing and had not been properly buried. Crop leaves (wheat, clover and ryegrass) occurred in smaller quantities than in the preceding month. The insects eaten increased in numbers, of these Weevils were the most important.

*April.*—The greater part of the food consisted of weed seeds; Polygonum aviculare, Polygonum Convolvulus, Stellaria media and Poa annua were present in largest numbers. A little seed corn (barley) was also taken. Insects occurred in about the same numbers as during the previous month.

*May.*—Insects, mainly Lepidopterous larvae, Click beetles and Weevils, were taken in increasing numbers. The basis of the diet consisted of weed seeds; the most important of these were Poa annua, Polygonum aviculare and Stellaria media. Some of the birds, killed

TABLE III.   *December* (13 *larks*).

| | Injuries | | | Benefits | | | Neutral | | |
|---|---|---|---|---|---|---|---|---|---|
| | Article of diet | Times occurred | Number occurred | Article of diet | Times occurred | Number occurred | Article of diet | Times occurred | Number occurred |
| **Seeds** | Wheat | 1 | 3 | Polygonum Convolvulus | 6 | 21 | — | | |
| | Trifolium pratense | 1 | 1 | Polygonum aviculare | 11 | 389 | | | |
| | Trifolium minus | 1 | 1 | Rumex Acetosella | 5 | 34 | | | |
| | | | | Chenopodium album | 8 | 303 | | | |
| | | | | Unidentified | 1 | 1 | | | |
| **Vegetation, &c.** | Wheat husk | 2 | little | Weed seed husk | 8 | moderate | Leaf (unidentified) | 1 | much |
| | Wheat leaf | 3 | much | | | | | | |
| | Clover leaf | 3 | moderate | | | | | | |
| | Rye-grass leaf | 5 | moderate | | | | | | |
| **Insects** | — | | | Weevils (Sitones) | 1 | 2 | — | | |
| **Miscellaneous** | — | | | — | | | — | | |

on spring sown corn, contained "small seeds" both clover and rye-grass (No. 162, Table II). In view of the numbers eaten it appears that larks are doing much damage in these localities.

*June.*—The diet consisted for the most part of weed seeds and injurious insects. The food of the young was almost entirely of insects (No. 211, Table II); this was particularly evident, as the old birds nearly always contained weed seeds. It is interesting to note that the turnip flea beetle (Haltica nemorum) was taken in numbers on several occasions by birds frequenting root fields.

*July.*—The food again consisted for the most part of weed seeds and injurious insects. Of the former Papaver Rhoeas and Polygonum aviculare were the most important, while of the latter Aphids, Pegomyia betae larvae and Weevils occurred in greatest numbers. In conjunction with the Pegomyia larvae mangold leaf was often taken.

*August.*—Weed seeds occurred in large numbers, the most important being Papaver Rhoeas and Polygonum aviculare. Insects occurred in smaller numbers, Halticid beetles being most plentiful.

Much corn (wheat, barley and oats) was taken from the stubbles after harvest.

*September.*—The food consisted almost entirely of weed seeds and Aphids. The latter, owing to the dry season, existed in large numbers on the swedes and had been eaten by birds which were killed in these localities (No. 314, Table II). Of the weed seeds Polygonum aviculare and Lychnis (?) occurred in large numbers. Some corn was also taken from the stubbles.

*October.*—Weed seeds formed the bulk of the diet; those occurring in greatest numbers were Papaver Rhoeas and Polygonum aviculare. Insects formed the remainder of the diet; of these Weevils and Dipterous pupae were the most important.

*November.*—Polygonum aviculare, Polygonum Convolvulus and Chenopodium album were the most important weed seeds occurring this month. These with a few grains of wheat from the autumn sowings and an occasional Dipterous pupa made up the diet. A number of clover seeds were found in birds which were killed on wheat, an adjoining field having been patched in the autumn with clover.

*December.*—69 °/₀ of the birds examined this month contained the leaves of some crop. The bulk of the diet consisted of weed seeds, chiefly Polygonum aviculare and Chenopodium album. On one occasion wheat was taken from the autumn sowings. The only insects that occurred were two Weevils (Table III).

TABLE IV.  LARKS.

| Month: | Average | January | December | November | October | September | August | July | June | May | April | March | February |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weather: | | Wet then frost and snow | Wet and warm | Fine with wet spells | Damp | Hot and dry then showers | Warm and dry | Hot and dry | Dry then showery | Warm and dry | Snow then warm | Fine warm | Fine cold |
| No. of stomachs from which calculated | 171 | 15 | 13 | 13 | 7 | 6 | 10 | 13 | 13 | 20 | 16 | 27 | 18 |
| **Seeds:—** | | | | | | | | | | | | | |
| *(a) Crop:—* | | | | | | | | | | | | | |
| Wheat | ·5 | 0 | 2·3 | 2·3 | 0 | 0 | 3·0 | ·8 | ·8 | 0 | 0 | ·7 | 0 |
| Barley | 1·1 | 0 | 0 | 0 | 0 | 3·3 | 4·0 | 0 | ·8 | 1·0 | 1·9 | 2·2 | ·5 |
| Oats | ·4 | 0 | 0 | 0 | 0 | 0 | 2·3 | 0 | 0 | 1·0 | 0 | 0 | 1·1 |
| Peas (?) | ·1 | 1·3 | 0 | 0 | 1·4 | 0 | 0 | 0 | 0 | ·5 | 0 | 0 | 0 |
| Vetch (?) | ·1 | 0 | 1·5 | 27·7 | 0 | 0 | 0 | 0 | 0 | ·5 | 0 | ·4 | 0 |
| Trifolium | 3·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10·5 | 0 | 0 | 0 |
| Lolium | 10·0 | 0 | 0 | 0 | 0 | 0 | 77·0 | 2·3 | 0 | 36·0 | 0 | 0 | 5·0 |
| Turnip (?) | 1·0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12·3 | 0 | 0 | 0· | 0 |
| *(b) Weed:—* | | | | | | | | | | | | | |
| Ranunculus | 4·2 | 0 | 0 | 0 | 0 | 0 | 0 | 21·5 | 29·2 | 0 | ·6 | 0 | 0 |
| Sinapis arvensis (?) | ·05 | ·7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fumaria officinalis | 1·2 | 0 | 0 | 0 | 0 | 0 | 5·0 | 16·1 | 3·8 | 0 | 0 | ·7 | 5·5 |
| Viola tricolor | 5·7 | ·7 | 0 | 0 | 0 | 0 | 0 | 50·0 | 0 | 47·0 | 3·1 | 0 | 1·7 |
| Papaver Rhoeas | 61·6 | 0 | 0 | 0 | 371·4 | 45·0 | 305·0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lychnis (?) | 3·9 | 13·3 | 0 | 0 | 0 | 0 | 1·0 | 0 | 0 | 0 | 0 | 9·6 | ·5 |
| Silene (?) | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 233·0 | 0 | 0 | 0 |
| Stellaria media (?) | 26·6 | 1·3 | 0 | 10·7 | 0 | 1·7 | 12·0 | 11·5 | 0 | 0 | 16·9 | 0 | 32·2 |
| Spergula arvensis | ·3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·9 | 0 | 1·7 |
| Sonchus oleraceus | ·1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·7 |
| Veronica | 1·0 | 0 | 0 | 0 | 0 | 0 | 12·0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polygonum aviculare | 157·6 | 130·0 | 299·2 | 273·1 | 54·3 | 75·0 | 37·0 | 33·8 | 19·2 | 204·0 | 279·4 | 186·3 | 300·0 |
| Polygonum Convolvulus | 12·2 | 10·0 | 16·1 | 26·9 | 7·1 | 16·7 | 0 | 12·3 | 11·5 | 9·0 | 15·0 | 13·3 | 8·9 |
| Polygonum lapathifolium | 3·0 | 2·0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5·5 | 1·9 | 23·7 | 3·3 |
| Rumex Acetosella | 13·6 | 18·0 | 26·1 | 7·7 | 22·8 | 8·3 | 13·0 | 4·6 | 2·3 | 11·5 | 1·2 | 4·1 | 50·5 |
| Rumex crispus | ·5 | 0 | 0 | 0 | 0 | 1·7 | 0 | 3·1 | ·8 | ·5 | 0 | ·4 | 0 |
| Chenopodium album | 35·5 | 53·3 | 233·1 | 95·4 | 20·0 | 1·7 | 0 | 0 | 1·5 | 412·0 | 10·0 | 5·5 | 2·8 |
| Poa annua | 40·2 | 1·3 | 0 | 0 | 20·0 | 1·7 | 0 | 15·4 | ·8 | 0 | 45·0 | 7·4 | ·5 |
| Unidentified | 12·7 | 3·3 | ·8 | 3·1 | 1·4 | 31·7 | 55·0 | 49·2 | ·8 | 0 | 1·2 | 1·8 | 3·9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VEGETATION, ETC.:—** | | | | | | | | | | | | | |
| *(a) Crops:—* | | | | | | | | | | | | | |
| Rye-grass, clover, etc. | 7·2 occ. | 2·2 occ. | 1·9 occ. | 1·0 occ. | ·8 occ. | 1·5 occ. | 1·0 occ. | 1·7 occ. | 0 | 1·5 occ. | 6·2 occ. | 6·0 occ. | 2·6 occ. |
| Wheat leaf | 1·1 occ. | 0 | 2·5 occ. | ·5 occ. | 0 | 0 | 0 | 0 | 0 | ·6 occ. | 2·3 occ. | 1·3 occ. | ·7 occ. |
| Oat leaf | 0 | ·4 occ. | ·6 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 occ. |
| Mangold leaf | 0 | 0 | 0 | 0 | ·8 occ. | 1·5 occ. | 0 | 0 | 0 | 0 | 0 | 0 | ·2 occ. |
| Swede leaf | 0 | 0 | 0 | 0 | 0 | ·8 occ. | 0 | 0 | 0 | 0 | 0 | ·7 occ. | ·07 occ |
| Wheat husk | 1·7 occ. | 3·3 occ. | 0 | ·5 occ. | ·8 occ. | ·8 occ. | 2·0 occ. | 3·3 occ. | 2·8 occ. | 1·5 occ. | 1·5 occ. | 0 | 1·0 occ. |
| Barley husk | 0 | 1·8 occ. | 3·7 occ. | 0 | ·8 occ. | 1·5 occ. | 2·0 occ. | 0 | 0 | 0 | 0 | 0 | 1·1 occ. |
| Oat husk | 0 | 0 | 0 | 1·0 occ. | 2·3 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·4 occ. |
| Lolium husk | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·08 occ. |
| Potato | 0 | ·4 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·03 occ. |
| *(b) Weeds:—* | | | | | | | | | | | | | |
| Thistle leaf | 2·8 | 0 | 0 | 0 | 0 | 0 | 2·0 occ. | 0 | 0 | 0 | 0 | 0 | ·1 occ. |
| Seedlings | 0 | 8·1 | 6·2 | 0 | 6·1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1·9 |
| Seed husk | 3·9 occ. | 6·3 occ. | 6·2 occ. | 6·0 occ. | 0 | 0 | 0 | 4·3 | 4·3 | 6·9 occ. | 6·1 occ. | 4·0 occ. | 3·6 occ. |
| Pos glumes | 0 | 0 | 0 | 3·5 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·3 occ. |
| Seed capsules | 0 | 0 | 0 | 0 | 1·5 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 occ. |
| Agrostis leaf | 0 | 0 | 0 | ·5 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·04 occ. |
| *(c) Neutral:—* | | | | | | | | | | | | | |
| Unidentified leaves | 2·2 occ. | 5·5 occ. | 5·0 occ. | 3·0 occ. | 4·6 occ. | ·8 occ. | 4·0 occ. | 5·0 occ. | 2·8 occ. | 4·6 occ. | ·8 occ. | 2·0 occ. | 3·5 occ. |
| **INSECTS:—** | | | | | | | | | | | | | |
| *(a) Beneficial:—* | | | | | | | | | | | | | |
| Earthworms' eggs | ·5 | ·4 | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Centipedes | 0 | 0 | 0 | 0 | 0 | 0 | 4·0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Ichneumon flies | 0 | 0 | 0 | 0 | 1·5 | 0 | 4·0 | 0 | 0 | 0 | 0 | 0 | ·5 |
| Staphylinid beetles | 1·1 | 1·5 | ·6 | ·5 | ·8 | 0 | 1·0 | 0 | ·8 | 0 | 0 | 0 | ·8 |
| Staphylinid beetle larvae | 0 | 1·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Carabid beetles | 0 | ·4 | 6·2 | 5·0 | 10·8 | 6·1 | 3·0 | 2·8 | 2·8 | 0 | 0 | ·7 | 3·0 |
| Dytiscid beetle larvae (?) | 0 | 0 | 0 | 0 | 0 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 |
| *(b) Harmful:—* | | | | | | | | | | | | | |
| Millipedes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Aphids | 0 | 0 | 0 | 5·5 | 47·7 | 39·2 | 1·0 | 450·0 | 0 | 0 | 0 | 0 | 45·3 |
| Cercopidae | 0 | ·4 | 0 | 0 | ·8 | 0 | 2·0 | 0 | 0 | 0 | 0 | 0 | ·3 |
| Earwigs | 0 | 0 | 0 | 0 | 13·8 | 23·1 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 |
| Pegomyia larvae | 0 | 0 | 0 | 0 | 0 | 0 | 15·0 | 15·0 | 4·3 | 0 | 0 | 0 | 4·3 |
| Bibio fly larvae | 0 | 1·8 | 0 | 9·0 | 0 | 3·1 | 0 | 0 | 2·9 | 0 | 0 | ·7 | ·06 |
| Lepidopterous larvae | 0 | 1·8 | 1·2 | 5·5 | 0 | 0 | 0 | 0 | 4·3 | 0 | 0 | ·7 | 2·5 |
| Ants | 0 | 0 | 0 | ·5 | 0 | 0 | 0 | 0 | 1·4 | 0 | 0 | 0 | 1·1 |
| Sawflies | 0 | 0 | 0 | 0 | 0 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Anobium beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 |

TABLE IV. *(continued).*

| Month:— | February | March | April | May | June | July | August | September | October | November | December | January | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weather:— | Fine-cold | Fine-warm | Snow then warm | Warm and dry | Dry then showery | Hot and dry | Warm and dry | Hot and dry then showers | Damp | Fine with wet spells | Wet and warm | Wet then frost and snow | |
| No. of stomachs from which calculated | 18 | 27 | 16 | 20 | 13 | 13 | 10 | 6 | 7 | 13 | 13 | 15 | 171 |
| **INSECTS:—** | | | | | | | | | | | | | |
| *Harmful (cont.):—* | | | | | | | | | | | | | |
| Wireworm | 0 | 0 | 0 | ·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·04 |
| Click beetles | 0 | 0 | 3·7 | 8·5 | 10·8 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | 2·0 |
| Chrysomelid beetles | 0 | 0 | 0 | 0 | 1·5 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Chrysomelid beetle larvae | | 0 | 0 | 0 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 |
| Weevils. Sitones | 2·2 | 6·3 | 14·4 | 10·5 | 13·8 | 11·5 | 3·0 | 11·7 | 21·4 | 1·5 | 1·5 | 2·0 | |
| Otiorhynchus | 0 | ·4 | ·6 | 0 | 0 | 0 | 0 | 0 | 1·4 | 0 | 0 | 0 | |
| Various | 0 | 0 | 0 | 5·0 | 6·2 | ·8 | 0 | 0 | 0 | 0 | 0 | 0 | |
| (total) | | 6·7 | 15·0 | 15·5 | 20·0 | 12·3 | | | 22·8 | | | | 9·6 |
| Halticid beetles | | ·7 | 0 | 1·5 | 41·5 | 2·3 | 33·0 | 6·7 | 2·9 | 0 | 0 | 0 | 7·3 |
| Gastropods (Helix) | ·5 | 0 | 0 | ·5 | 1·5 | ·8 | 0 | 0 | 0 | ·8 | 0 | 0 | ·4 |
| *(c) Neutral:—* | | | | | | | | | | | | | |
| Spiders | 0 | 3·0 | ·6 | 2·0 | 2·3 | 0 | 2·0 | 1·7 | 1·4 | 0 | 0 | 0 | 1·1 |
| Collembola | 0 | 0 | 0 | 1·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Stratiomyid fly larvae | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·05 |
| Muscid flies | 0 | 1·5 | 0 | ·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·2 |
| Gnats | 0 | 0 | 0 | 0 | 0 | 4·6 | 1·0 | 0 | 0 | 0 | 0 | 0 | ·1 |
| Pupae cases | 0 | 0 | 0 | ·5 | 0 | 0 | 4·0 | 5·0 | 24·2 | 6·1 | 0 | 2·3 | 4·1 |
| Dipterous larvae | 0 | 0 | 0 | 0 | 0 | 0 | 2·0 | 1·7 | 0 | 0 | 0 | 0 | ·3 |
| Silphid beetles | 0 | 0 | ·6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·05 |
| Scarabid beetles | 0 | ·4 | 0 | ·5 | 0 | 0 | 0 | 0 | 1·4 | ·8 | 0 | 0 | ·3 |
| Beetle remains | 0 | 0 | 0 | 0 | ·8 occ. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ·06 occ. |

*January.*—The food consisted mainly of weed seeds; those which occurred in largest numbers were Polygonum aviculare, Chenopodium album and Rumex Acetosella. 73 $°/_{o}$ of the birds examined contained the leaves of some crop. Extremely few insects occurred.

## TABLE IV.

This table is a comparison of the monthly food in detail, being compiled in the same way as in the case of the starling.

To mention briefly a few of the facts shown by this table:—

Corn is taken at the times of seeding, but not in sufficient quantities to be of importance; much is however taken from the stubbles after harvest, but this is not of much economic value. The number of clover and rye-grass seeds taken in the spring and autumn indicates an injury to this crop. With regard to weed seeds, it will be noticed that only a small number of species occur with any regularity; thus indicating that the lark exercises a special preference for some kinds. Its fondness for seeds of the Polygonaceae is particularly evident. Some species of seeds are taken only at certain times of the year, for example, Poa annua, Polygonum lapathifolium and Papaver Rhoeas. The weed seed figures for June are rather low on account of the inclusion of a number of young birds. The consumption of crop leaves is for the most part confined to the winter months.

Very few beneficial insects are taken, Carabid beetles alone occur with any regularity. Of the harmful insects Aphids, Pegomyia larvae and Halticid beetles occur only in the summer, Click beetles only in the spring, while Weevils are taken regularly, although more occur in the summer months.

Although not shown in the table, the amount of grit found in the gizzards also varied with the time of year, more being present in the winter than in the summer months.

By looking at the last column in Table IV it will be seen that weed seeds are far and away the most important article of diet, harmful insects come next, with crop leaves and "small seeds" forming a small proportion.

The food of the lark may be summed up as follows:—The bulk of the food consists of weed seeds; this is eked out in the summer months by insects, and in the winter by pieces of leaf, for the most part of crops.

Both Saunders (15) and Newton (13) state that there is a migration of larks to this country in the autumn from Northern Europe and a return in the spring. From this it would follow that the damage to

TABLE OF VARIOUS BIRDS (1).

| No. | Bird | Date | Time | Place | Locality | Weather | Sex | Injuries | | Benefits | | Neutral |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Crop | Insects, etc. | Weeds | Insects, etc. | |
| 5 | Sparrow | Feb. 4th | 11 a.m. | Lowestoft | New ley | Damp | ♀ | 14 whole and 56 part grains of barley | — | 5 Rumex seeds | — | \| |
| 30 | ,, | March 20th | | ,, | Barley | Fine | ♂ | 40 part grains of wheat | — | | — | \| |
| 6 | Greenfinch | Feb. 4th | 11 a.m. | ,, | New ley | Damp | ♂ | 54 part grains of barley | — | 1 seed of Polygonum Convolvulus | — | \| |
| 7 | ,, | ,, | ,, | ,, | ,, | ,, | ♂ | 42 part grains of barley | — | 1 seed of Brassica Sinapis (?) | — | \| |
| 379 | ,, | Dec. 4th | 6 p.m. | Cambridge | | Dull | ♂ | | — | 12 seeds of Brassica Sinapis (?) 38 seeds of Polygonum aviculare | — | \| |
| 78 | Chaffinch | March 22nd | 11 a.m. | Brampton, Norfolk | Barley | Warm | ♀ | Many small pieces of barley grain | — | | — | \| |
| 389 | Yellow Ammer | Dec. 7th | | Cambridge | Wheat | Wet | ♀ | 6 grains of wheat and a little husk | 1 Staphylinid beetle | 1 seed of Polygonum aviculare | — | \| |
| 1 | Lapwing | Feb. 2nd | | Brampton, Norfolk | | | ♂ | | 2 Earthworms | | — | \| |
| 91 | Waterhen | March 26th | 3 p.m. | ,, | Meadow | Cold | ♀ | | — | 1 seed (unidentified) Large amount of Elodea shoots and rushes | — | \| |
| 10 | Blackheaded Gull | Feb. 4th | noon | ,, | Flying | Fine | ♀ | | 10 Earthworms 2 Centipedes 1 Carabid beetle 1 Staphylinid beetle larva | | 7 Gastropods [2 Limnaes, 5 Planorbis (?)] 2 Lepidopterous larva (Noctua) 8 Wireworms | \| |

| No. | | Date | Time | | | | Sex | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 65 | „ | March 13th | „ | Earl's Colne, Essex | Near sheep fold | Wet | ♀ | — | 5 Earthworms | 1 seed of Polygonum aviculare / 1 seed of Polygonum Convolvulus / 1 seed of Chenopodium album | 1 Click beetle | — |
| 148 | Pigeon | April 26th | — | — | — | — | — | Many pieces of swede (?) leaf / 69 grains of wheat / 137 grains of barley / 82 milled and 6 unmilled sainfoin seeds | — | 2 seedheads of Veronica and 3 pieces of leaf | — | — |
| 244 | Turtle-dove | June 26th | 4 p.m. | Earl's Colne, Essex | Turnip seed | Rain | — | 69 turnip seeds | — | 630 seeds of Fumaria officinalis / 19 seeds of Polygonum aviculare | — | 1 Spider |
| 246 | „ | June 27th | 11 a.m. | „ | „ | After rain | — | 2150 turnip seeds | — | 17 seeds of Fumaria officinalis | 1 Gastropod (Helix) | 1 Dipterous larva |
| 313 | „ | Sept. 14th | 7.30 a.m. | „ | — | Wet | — | 200 grains of wheat and some husk | — | 5 seeds of Polygonum aviculare / 6 seeds [unidentified] | — | — |
| 133 | Black-bird | April 8th | — | Suffield, Norfolk | Spring wheat | Cold | ♀ | 1 grain of oats and a little husk | — | — | 1 Wireworm / 1 Click beetle / 1 Weevil | — |
| 134 | „ | „ | — | „ | „ | „ | ♂ | — | 4 Earthworms / 2 Staphylinid beetle larvae | — | 5 Wireworms / 3 Click beetles | 1 Dipterous larva |
| 256 | „ | July 16th | 10 a.m. | Brampton, Norfolk | Garden | Hot | ♀ | 6 gooseberry skins and some seeds | 1 Centipede | — | 1 Sawfly | 1 Beetle [unidentified] / A piece of grass |

TABLE OF VARIOUS BIRDS (2).

| No. | Bird | Date | Time | Place | Locality | Weather | Sex and remarks | Injuries | | Benefits | | Neutral and Miscellaneous |
| | | | | | | | | Crops | Insects, etc. | Weeds | Insects, etc. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 48 | Rook | March 6th | — | Univ. Farm, Camb. | Spring wheat | Fine | ♀ | 60 grains of wheat 4 peas (about) | 2 Staphylinid beetle larvae | 1 seed (Compositae) | — | Small pieces of bone, with little flesh |
| 49 | ,, | ,, | — | ,, | ,, | ,, | ♀ | 70 grains of wheat and large amount of wheat husk 2 grains of oats 1 grain of barley and a little barley husk Little pea husk | — | — | — | — |
| 50 | ,, | ,, | — | ,, | ,, | ,, | ♀ | 85 grains of wheat 12 grains of barley 4 peas 1 young wheat sprout Much husk of wheat, barley and peas | — | — | — | — |
| 53 | ,, | March 10th | — | ,, | Newly sown barley | ,, | ♀ | 4 small pieces of pea Much barley husk | — | — | — | — |
| 54 | ,, | ,, | — | ,, | ,, | ,, | ♀ | 40 grains of barley 4 small pieces of pea Much barley husk | — | — | — | — |
| 56 | ,, | ,, | — | ,, | ,, | ,, | ♀ | 25 pieces of pea and husk 1 oat grain Much oat husk | 1 Staphylinid beetle larva | — | — | — |

TABLE OF VARIOUS BIRDS (3).

| No. | Bird | Date | Time | Place | Locality | Weather | Sex | Injuries | | Benefits | | Neutral |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Crops | Insects, etc. | Weeds | Insects, etc. | |
| 62 | Rook | March 12th | 10 a.m. | Earl's Colne, Essex | Spring wheat | Wet | ♂ | 7 grains of barley and a large amount of barley husk | 6 Staphylinid beetle larvae | — | — | — |
| 106 | ,, | April 3rd | 11 a.m. | Marsham, Norfolk | Newly sown barley | Fine | ♂ | 110 grains of wheat 1 grain of barley and large amount of wheat and barley husk | 3 Earthworms | 2 seeds of Polygonum aviculare | 1 Noctua larva 2 Wireworms 1 Fly pupa ((?) Anthomyid) | 3 Fish vertebrae Lump of fat with little muscle 2 pieces of grass |
| 168 | ,, | May 12th | 7 p.m. | Earl's Colne, Essex | Rookery | Dry | young | 14 small pieces of bean Much barley husk | — | — | — | — |
| 185 | ,, | May 22nd | noon | Marsham, Norfolk | ,, | Fine | ,, | 20 grains of maize and much husk | — | — | — | — |
| 186 | ,, | ,, | ,, | ,, | ,, | ,, | ,, | Many stamens of oak with pedicels | — | — | 20 Cynipid galls on oak 1 Weevil (Otiorhynchus) | 1 Mayfly (Ephemera) 2 Stratiomyid fly larvae |
| 187 | ,, | ,, | ,, | ,, | ,, | ,, | ♂ | Many stamens of oak with pedicels | — | — | 23 Cynipid galls on oak | 2 Fish vertebrae |
| 64 | Jackdaw | March 13th | noon | Earl's Colne, Es-ex | Sheep fold | Wet | ♀ | 30 grains of oats and much husk Few pieces of cut hay and straw | — | — | — | — |

leys and wheat is done for the most part by the migratory birds; but the damage to "small seeds" is probably done by the birds breeding here.

The conclusion reached is that, on the whole, the lark is beneficial; but, owing to the injuries done at certain times of the year, there is no reason why it should be specially protected, although its wholesale slaughter is to be deprecated.

## PART IV. VARIOUS BIRDS.

The records of a few other birds, which were obtained at various times during the year, are given above.

An insufficient number have been obtained for an opinion to be expressed as to their utility, but it was thought that the details might be of use to others interested in the subject. In the case of rook No. 106, the wheat was probably taken from a stack, as these birds were known to have attacked one in the district.

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NOTE. Since the above was written, Laura Florence has published a paper on "The Food of Birds" (*Trans. High. and Agric. Soc.* 1912) in which the crop contents of 616 birds of various species, mostly from the North East of Scotland, are described.

# ON OVARIOTOMY IN SOWS WITH OBSERVATIONS ON THE MAMMARY GLANDS AND THE INTERNAL GENITAL ORGANS.

By K. J. J. MACKENZIE, M.A. and F. H. A. MARSHALL, M.A.

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In the paper which follows an account is given of our observations upon the ovaries and mammary glands in the sow and on the relation subsisting between these organs. This is followed by remarks on the prevalent method of spaying sows. In a subsequent paper we hope to state the results of our experiments dealing with the influence of ovariotomy upon growth and fattening.

## PART I.

Before sexual maturity the mammary glands of all animals as far as is known are in a very rudimentary condition. With the onset of puberty there is a distinct growth of gland tissue accompanied by an increase of ducts. This breast hypertrophy, as is well known, is very pronounced in women, but the growth in this case is due largely to a deposition of fat. Apart from the pubertal growth which is more or less permanent there is known frequently to be a swelling of the mammary glands at menstruation or in animals at heat. Thus women often experience a fulness and tenderness of the breasts due to increased vascularisation at such times. In one of the lower types of mammals, the marsupial cat, Hill[1] has described the growth and congestion of the mammary glands at the heat periods as being so great as frequently to have misled him into supposing that the animals were pregnant, but the breast growth which takes place at heat or

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[1] Hill, *Quart. Jour. Micr. Sci.*, vol. LVI. 1910.

menstruation is generally slight in comparison with the great hypertrophy which the glands undergo during pregnancy, as a preparation for the secretion of milk.

It is this great hypertrophy which led Starling and other physiologists to perform experiments with a view to determining the precise source of the stimulus for mammary growth in pregnant animals. Ribbert[1] and Pfister[2] had already shown that the functional correlation between the generative organs and mammary glands was almost certainly chemical and not nervous, since portions of transplanted glands in rodents were found to undergo hypertrophy during pregnancy, and secretion after parturition, in spite of the fact that they had been grafted in abnormal positions so that their usual nerve connections were completely severed.

Such considerations as these suggested to Lane-Claypon and Starling[3] that the growth of the mammary glands depended upon the action of one or more internal secretions or hormones elaborated either by one of the generative organs, or by the foetus itself, or by some combination of organs. They accordingly proceeded to inject extracts of ovary, of uterus, of placenta, and of foetus, into virgin rabbits in which the mammary glands were believed to be altogether undeveloped. Foetal injections alone were found to bring about breast hypertrophy, but the amount of extract employed had to be very large before any result could be appreciated. As a result of these experiments, Lane-Claypon and Starling came tentatively to the conclusion that breast hypertrophy results from the action of a specific hormone elaborated by the foetus. Foà[4], and Biedl and Koenigstein[5] have carried out similar experiments and arrived at like conclusions.

Basch[6] has performed injection experiments which lead him to conclude that breast hypertrophy and milk secretion are due to the combined action of the ovaries and placenta, but since the animals experimented on were all (with one exception) multiparous, the evidence cannot be regarded as convincing.

All the above mentioned investigators have been recently criticised by Frank and Unger[7] who have shown (which has also been observed by

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[1] Ribbert, *Arch. f. Entwick.*, vol. VII. 1898.

[2] Pfister, *Beitr. z. Geb. u. Gynäk.*, vol. V. 1901.

[3] Lane-Claypon and Starling, *Roy. Soc. Proc.*, B, vol. LXXVII. 1906.

[4] Foà, *Arch. di Fis.* vol. V. 1908.

[5] Biedl and Koenigstein, *Zeits. f. exp. Path. u. Therapie*, vol. VIII. 1910.

[6] Basch, *Monatsschr. f. Kinderheitk.*, vol. VIII. 1900.

[7] Frank and Unger, *Arch. International Medicine*, vol. VII. 1911.

Ancel and Bouin[1]) that the rabbit's mammary glands may undergo growth change independently of pregnancy. These changes are believed to be cyclical and to occur under the influence of the ovary. Injection of foetal extract did not confirm Starling's results, but ovarian injections appeared to produce hypertrophy. The experiments were upon rabbits and rats.

Moreover Ancel and Bouin state that destruction of all the corpora lutea in the ovaries of pregnant rabbits has the effect of arresting the development of the mammary glands. This result receives some confirmation in O'Donoghue's[2] observations upon the marsupial cat, in which he finds a close parallelism between the growth of the mammary glands and the development of the corpora lutea. It would seem probable, therefore, that the ovaries play some part in the mechanism of mammary development and milk secretion.

Lastly, according to Ott and Scott[3], and Schäfer and Kenneth Mackenzie[4], injection of corpus luteum extract in lactating animals causes almost immediately an increased secretion of milk.

### The Mammary Glands in Spayed and Open Sows.

Our own observations upon sows confirm the view that the ovaries are an essential factor in mammary growth, or at any rate in such mammary growth as occurs in non-pregnant animals.

In black pigs the matter is complicated by the occurrence of melanic pigment in the mammary region, but, as will be described below, this pigment, so far as we have been able to determine, is not derived from extravasated blood and has no connection with the occurrence of heat. Apart, however, from this black pigment the mammary tissue of two black sows killed during the prooestrum or oestrus showed some indications of increased vascularisation.

In Middle White sows the condition of the mammary glands was markedly different in open (*i.e.* unoperated) animals from what it was in pigs which had been spayed. Thus in four fully grown sows whose ovaries had been removed when young there was no trace whatever of any growth of the breasts at the time of killing. On the other hand in four open animals of the same age and kept under identical conditions,

[1] Ancel and Bouin, *Jour. de Physiol.*, 1911.

[2] O'Donoghue, *Quart. Jour. Micr. Sci.*, vol. LVII. 1911.

[3] Ott and Scott, *Proc. Soc. Exp. Biol.*, N. Y. 1910.

[4] Schäfer and Mackenzie (K.), *Roy. Soc. Proc.*, B, vol. LXXXIV. 1911.

but not allowed to breed, there was a definite development of gland tissue which could be seen with ease superficially after the sows were killed and on cutting into the abdominal region. Moreover, in one of these sows there was a slight but definite secretion of a thin milky fluid which was probably colostrum. This latter condition is of course unusual in animals which have not bred, although cases have been recorded in which virgin animals of different kinds have yielded milk, and the same is stated to have been the case occasionally in male animals.

In another Middle White sow, which was killed when on heat, there was a pronounced congestion of the mammary glands, the blood vessels appearing to be both larger and more numerous than at other times. Sections through portions of these glands are shown in Plate VI, figs. 1 and 2. Whether or not any of the blood corpuscles were actually extravasated in the tissue could not be confidently determined, but certain of the histological appearances supported this view.

In a sucking pig killed many weeks before puberty there was no breast hypertrophy.

In view of these observations it is clear that there is a close correlation between the ovaries and the mammary glands; and further, there is evidence that the mammary glands undergo cyclical changes corresponding with those which take place in the internal generative organs during the oestrous cycle.

*Practical Considerations.*

In the case of swine the matter becomes one of economic importance. There is a very great prejudice against using the flesh of animals that at time of slaughter have been on "heat" or which show signs of coming on or going off "heat." This is more so in the case of flesh required for curing or for bacon than for meat which is to be sold as pork; though even in this latter case the prejudice is very great. It is stated that the flesh of pigs slaughtered under these conditions is not only difficult to cure but is also, when converted into bacon, very much inferior in taste. This prejudice is so great that one large firm of bacon-curers lose some thousands a year through it. They have not sufficient accommodation, and it would interfere too greatly with their system of paying for each consignment of pigs by weight, to allow sows showing signs of oestrus to be kept back until all traces of the condition had disappeared. Their practice is to have the carcase of each

sow examined by means of an incision made with an ordinary butcher's knife, into the tissue of the "belly-piece," *i.e.* the abdominal region. The examiner being very experienced—for the number of swine dealt with is very great, and he is employed in examining day after day— it is believed that he is able to pick out all the carcases of sows slaughtered at or about the period of oestrus. Such sows cause a double loss to the industry. In the first place each "side" has a strip cut off the belly. In a normal case the strip so removed would be converted into a first quality "cut" of bacon, but owing to the condition of the carcase, due, the manufacturers believe, to the changes associated with heat, it is consigned to the offal tub. The Managing-Director of the firm in question informed us that in the case of 3267 sows the amount of flesh so cut off averaged 3·75 lbs. in weight per pig, and that this meat loses 70 per cent. in value. We have not yet had an opportunity of determining exactly what tissues are so removed; but it is obvious from the most casual inspection, that the strip involves a large part of the mammary area.

The wastage through the removal of this part of the side is further augmented by a decreased value of the whole carcase. For the "sides" so mutilated have to be kept apart, and after undergoing the process of curing are sold as *unbranded* bacon. Such flesh is sold approximately at £10 per ton less than the *branded* produce; or stated in other words there is a further loss of some 7*s.* on the carcase of every sow killed at or about the period of heat. The firm in question deals with some hundred thousand pigs in a year so that the loss is a very serious one, and we have evidence of further losses in the industry elsewhere[1].

### *The Ovarian Cycle in Pigs.*

Heat in pigs lasts four or five days, this period including the prooestrum, or time of preparation, and oestrus, or the time when the sow is ready to receive the boar. During the prooestrum, which lasts for perhaps two or three days, the uterus and generative tract become congested and, as already mentioned, the congestion may also extend to the mammary glands. The ovaries at this time contain numerous Graaffian follicles, which from their degree of protrusion are evidently in a state of almost complete maturity. Ovulation, however, does not take place until

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[1] Messrs C. and S. Harris of Calne inform us that out of some 1400 sows 11·19 % were found by them to show what their skilled assistants believed to be signs of having died *in oestro*.

oestrus, or the period of actual desire (or at any rate not until a late stage in the prooestrum). Thus in two Large Black sows which were killed when on heat the uterus in each case was enlarged and congested, but the ovaries contained no corpora lutea. On the other hand a number of large follicles were present, thereby showing that ovulation had not yet taken place. In another black sow, killed five days after signs of prooestrum had been noticed, it was found that ovulation had not even then occurred, though there were numerous protruding follicles but no ruptured ones. In a Middle White sow killed a week after heat was first noted, ovulation had taken place and there were numerous developing corpora lutea in each ovary. In another white pig killed during heat (and almost certainly during oestrus) ovulation had just occurred, as was apparent from the presence of newly ruptured follicles. It is clear therefore that the beginnings of mammary growth are not due to a nervous reflex set up by ovulation. Moreover, these observations bear out the statement by Sanders Spencer[1] that it is best for sows to be served late rather than early in the heat period, and that if they are put to the boar too soon they are liable to be barren to the service. It would seem probable, if not certain, that union between the ova and spermatozoa is most easily secured when the latter are introduced into the female generative passages at about the same time as the liberation of the ova, and consequently that if copulation precedes ovulation by too considerable an interval the chances of the ova becoming fertilised are much reduced.

Ovulation in the sow can take place independently of the occurrence of coition, for in the case of the sows mentioned above in which the follicles had ruptured, this had happened in the absence of the boar. Spontaneous ovulation was also noted to have occurred in a Large Black sow. It would appear therefore that Hausmann's statement[2] that ovulation in pigs is consequent upon coition is incorrect. In ovulating spontaneously during oestrus the sow resembles the sheep (at least ordinarily), the mare, and the bitch, but differs from the rabbit and the ferret[3], for in the two last mentioned animals the follicles in the ovary do not generally rupture excepting after the reflex stimulus set up by sexual intercourse. Since sows are capable of

[1] Sanders Spencer, *Pigs, Breeds and Management*, 2nd edition, London, 1898. This is what he says:—"We have found that sows are most likely to prove in pig when mated at the later part of the period of oestrum."

[2] Hausmann, *Ueber die Zeugung und Entstehung des wahren weiblichen Eies*, Hanover, 1840.

[3] For references, see Marshall, *The Physiology of Reproduction*, London, 1910.

ovulating independently of any such stimulus it ought to be possible to induce pregnancy by artificial insemination.

### *Further Practical Considerations.*

Another matter of practice may conveniently be considered here. Notwithstanding the fact that many observant pig-breeders, Sanders Spencer among the number, advise to the contrary, it is a very prevalent practice to run the boar loose among a considerable number of non-pregnant or empty sows, in a yard. Observation shows that the boar when first turned in among the herd will serve the sows first coming into use several times; copulation taking place at nearly all stages of the heat period. Subsequently the boar may refuse sows that are evidently willing to be served, though if it so happens that the heat period does not occur among the sows in the herd for some little time, the boar after the period of rest will regain his sexual activity and, in the case of the first sow or sows coming on heat, his powers will once again be wasted by an excessive number of matings, many of which will take place in the comparatively early stages of heat. It is obviously better, if as shown above it seems desirable that copulation should take place at one particular period of the ovarian cycle, to keep the boar apart from sows until the right moment for mating occurs. Our investigation fully confirms the experience of those who have deprecated the practice just described. Pigmen who assure their masters to the contrary are obviously prejudiced. For it must be pointed out that when they affirm that the only way to make sure of sows being in pig is to allow free intercourse with the boar they also counsel a practice which saves themselves considerable trouble.

### *Black Pigment in the Mammary Glands of Sows.*

The mammary tissue in black pigs often contains a quantity of melanic pigment which is deposited more especially in close contiguity to the epithelial cells lining the ducts, but whether it is actually intercellular could not be determined. It may also occur in the connective tissue between the ducts, sometimes quite deep down embedded apparently in the fat of the abdomen. There is no evidence that the black pigment is derived from extravasated blood as one might readily suppose from the state of congestion of the mammary glands at the time of heat. This is shown by the Prussian Blue reaction. Sections

which were treated with a mixture of one per cent. hydrochloric acid and one per cent. potassium ferrocyanide and then warmed did not show the characteristic blue stain which indicates the presence of iron. Attempts to detect iron by dissolving up some of the mammary tissue in a test tube and then testing for its presence were likewise negative in result. Thus there was no evidence that iron was contained in this pigment which must be regarded therefore as entirely different from the black pigment found in the uterine mucous membrane of sheep at the end of the sexual season.

Moreover, black pigment in some quantity was found in a young Large Black pig in which the internal generative organs were quite infantile, as well as in another pig of the same breed in which the Graaffian follicles were only just beginning to ripen, and in another black pig which had been spayed. These three animals presumably could none of them have experienced heat and therefore the pigment could not have been derived from prooestrous extravasation.

In addition to these sows pigment in greater or less quantity was observed in the mammary region in five others. It was present also in a small quantity in a hog. Furthermore, Messrs Harris of Calne, and Mr Hasler of Dunmow, have remarked to us on its presence. The former though stating it to be present more frequently (or almost solely) in sows sent us bacon cut from the mammary region of a hog in which this pigment was undoubtedly present in association with the rudimentary mammary ducts. The occurrence of the pigment is much deprecated by bacon curers since it renders the cut commonly known as the " streaky " or " belly piece " discoloured and " seedy" in appearance.

Whether the black pigment above described is present equally in all breeds of black pigs is a point that requires investigation. So far as our experience has at present gone we only failed to find it in one animal. This was a pedigree Large Black sow which was almost mature but had not been on heat at the time of killing.

In twelve white sows (seven of which were mature and unoperated) we failed to discover any black pigment.

These observations indicate that the pigment in black pigs is probably similar in kind to the melanic pigment of the hair. The case is perhaps comparable to what is known to occur in the Silky Fowl in which the dark colour of the skin, comb, and wattles is correlated with deposition of pigment in the mesenteries and other internal parts of the body[1].

[1] Bateson and Punnett, *Jour. of Genetics*, vol. I. 1911.

*Spaying as ordinarily practised.*

The operation of spaying as customarily carried out by non-professional gelders includes the removal of the uterus and Fallopian tubes as well as the ovaries, or expressed in surgical terminology it consists of double ovariotomy combined with complete hysterectomy.

Physiologically there can be no reason for removing the uterus, since animals are completely de-sexed by extirpating the ovaries alone, these being the essential organs of reproduction, whereas the uterus, although necessary in mammals, is an accessory organ. Further it has been shown experimentally in rodents that whereas the uterus atrophies (or if the operation is performed before puberty, remains infantile) after double ovariotomy, the ovaries on the other hand are unaffected by hysterectomy and continue to display their normal functional activity. Consequently, removal of the uterus without removal of the ovaries does not lead to the cessation of the oestrous cycle.

Probably in the ordinary commercial spaying of sows it would be impracticable to remove the ovaries without the uterus, for whereas the latter organ in young pigs is readily felt and recognised by the operator the ovaries are very minute and seem merely to be, so to speak, incidentally involved in the process of removal. But, as has just been shown, the view (which practical gelders and others appear commonly to hold), that it is primarily essential to extirpate the "bed" has no scientific basis. Fortunately for the gelder removal of the uterus has no detrimental results, but physiologically speaking it is immaterial whether this organ is removed or not.

On the other hand it is absolutely essential if the recurrence of heat is to be prevented that both ovaries should be removed entire. If in the process of extirpation the uterus is torn away in such a fashion as to leave one ovary (or only a portion of one ovary) behind, and if it retains its vascular connections and so is not starved, there is every reason for believing that this retained ovarian tissue will continue to function and the oestrous cycle continue to occur. We would suggest that in those cases in which heat is said to be experienced after spaying this has been due to the operation having been imperfectly carried out. If this suggestion is correct the result is comparable to those human cases in which menstruation has taken place after the supposed removal of both ovaries[1].

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[1] For descriptions of experiments and references to literature, see Marshall, *The Physiology of Reproduction*, London, 1910.

At the present time, and indeed for some years past, notwithstanding the losses to the bacon manufacturers described above, and the inconvenience and delay caused to pork butchers by deferring slaughter till the period of "heat" has passed, and in spite of a belief among pig feeders that spayed sows feed quicker and better than "open" ones, the practice of ovariotomy is on the decline. In certain large districts, where swine are a very important commercial consideration to the local husbandry, spaying has largely disappeared.

The operation has certainly been known as an ordinary occurrence on the farm for a very long time. We find it referred to by Aristotle in his *Historia Animalium*[1], which was written about 340 B.C. This is what he says: "The ovaries of sows are excised with the view of quenching in them sexual appetites and of stimulating growth in them in size and fatness. The sow has first to be kept two days without food, and after being hung up by the hind legs, it is operated on; they cut the lower belly, about the place where the boars have their testicles, for it is there that the ovary grows, adhering to the two divisions (or horns) of the womb; they cut off a little piece and stitch up the incision."

In this country the practice of spaying sows dates back at least to the eighteenth century and probably very much further. In the *Annals of Agriculture*[2] a writer gives an account of a pig feeding experiment carried on from December 1785 to January 1786, in which he mentions a "cut" sow without any reference to the condition being uncommon. He further refers to the difference in weight between a "cut" and an "open" sow.

In the *Complete Grazier*[3], by a Lincolnshire farmer, published in 1808, the author tells us that sows used for breeding *after the age of six years* "may be spayed and put up to fatten." In Loudon's *Encyclopaedia of Agriculture*, 1825, we learn on p. 1018, that "such of the pigs of both sexes as are not to be kept for breeding, are usually castrated or spayed when about "a month old." We also get a description of the operation of spaying in the General Index. Notwithstanding the reluctance among agriculturists to abandon practices sanctioned by long tradition the decadence of commercial ovariotomy is an admitted fact. One explanation however is the great change that has come over the system of pig-feeding in this country. In these days the great majority of pigs are slaughtered before they are nine months old; this is more

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[1] Aristotle, *Historia Animalium*, Thompson's Translations, Oxford, 1910.
[2] *Annals of Agriculture*, vol. VI. (p. 231), 1786.
[3] *Complete Grazier* (p. 62), 1808.

especially the case with bacon pigs. Fifty or sixty years ago very few pigs were killed before attaining one year and the majority of bacon hogs lived till they were 18 months old or more. Obviously the loss—if loss there be—which takes place during the growing and fattening periods, due to the excitement and derangement of the animal's system each time that it is on heat, will be greater in the case of the older animals. Actual experiment has not yet determined the fact of this loss occurring, but it is a matter of somewhat general belief among pig feeders and is one of the matters included in our investigation. Another reason for the decline of the practice of spaying is the slight risk which the animals run at the hands of the operator, and yet another is the fact that many feeders aver that they find some "spayed" sows which behave as if they were "open." It is argued therefore that the risk and slight expense of the operation are futile. This experience is so frequently reported that it seems only too likely that faulty operating is not uncommon. This is a point regarding which we hope to obtain definite information.

### *Summary of Practical Conclusions.*

(1) Distinct changes take place in the tissues of the mammary region in sows during the period of heat. These changes consist mainly of congestion of the glands. Moreover the part affected is in the pig of much commercial value.

(2) The black pigment often found in the mammary tissue of black sows is not associated with the recurrence of heat, and the bacon manufacturer who wishes to differentiate between sows slaughtered at this period and other sows must not rely on this pigment as a guide.

(3) Since ovulation occurs during oestrus and not during pro-oestrum it is advisable to "hog" the sow towards the end of her heat period.

### DESCRIPTION OF PLATES.

Plate VI, Fig. 1. Section through small portion of mammary tissue from a Middle White sow killed in a condition of prooestrum. *a.* duct, *b.* blood corpuscles in enlarged vessels in the neighbourhood of the ducts. Some of the corpuscles however appear as though they might have been extravasated.

Fig. 2. Section through small portion of mammary tissue from a Large Black sow. *a.* ducts with black pigment in and near their walls, *b.* fat cells from which the fat had been dissolved out.

Fig. 3. A portion of the same section as that shown Fig. 2, but more highly magnified. Lettering as in Fig. 2.

Plate VII, Figs. 4 and 5. Photographs of pieces of bacon cut from the mammary region of Large Black sows, showing large quantities of black pigment seen as small black dots or specks.
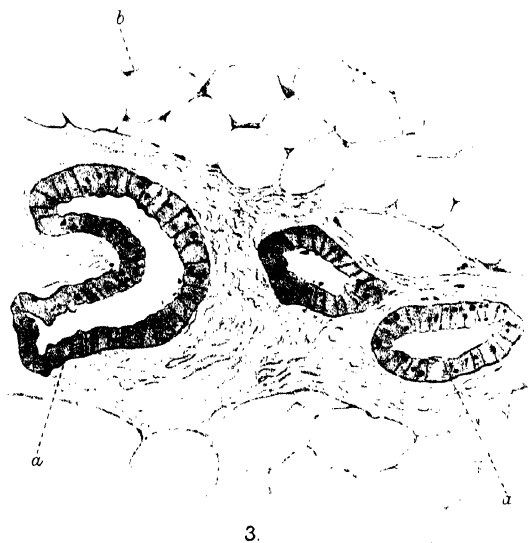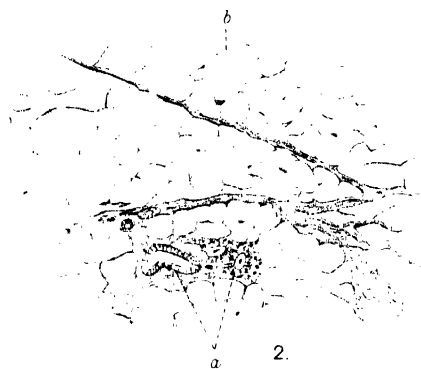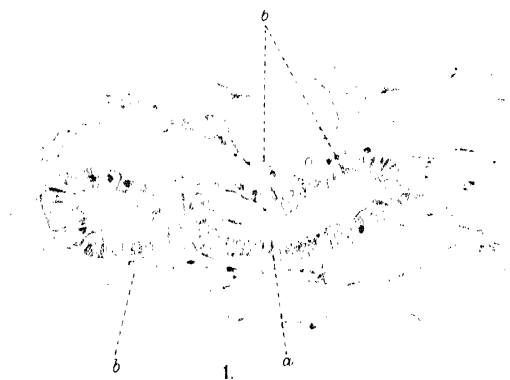
1.

2.

3.

Fig. 4.



Fig. 5.

# STUDIES IN THE INHERITANCE OF DISEASE RESISTANCE. II.

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THE $F_2$ generation of crosses between a number of varieties of wheat susceptible to the attacks of Yellow Rust (*Puccinia glumarum*) and "American Club" which shows an extraordinary degree of resistance to the attacks of this parasite showed in the year 1906 obvious segregation into immune and susceptible forms. The immunity was further found to be independent of any recognizable morphological feature. An analysis of these $F_2$ generations demonstrated the fact that the immune plants were present to the extent of 25 per cent., that is that the property of resisting the attacks of Yellow Rust behaves as a recessive character to the property of being susceptible[1]. The plants which proved susceptible showed, however, very varied degrees of susceptibility. Many were as covered with rust pustules as Michigan Bronze, one of the extremely susceptible varieties used as a parent. Others on the other hand were relatively slightly attacked and between these extremes all degrees of susceptibility appeared to exist. Nilsson-Ehle who has also observed the segregation of this pair of characteristics has also noted the wide differences in susceptibility in plants of the $F_2$ and succeeding generations[2].

Since 1907 investigations have been carried on to determine whether the extracted immune types would breed true to the feature of immunity and to attempt to find some explanation of the wide range of susceptibility shown by a portion of the $F_2$ individuals. For the most part the descendants of the cross American Club × Michigan Bronze were used for this purpose since the parents showed disease resistance and susceptibility to a greater extent than any others.

In order to test the immunity as thoroughly as possible two sowings of grain were made in autumn and spring respectively of each wheat-year. By sowing late in the spring it was possible to obtain young

[1] Biffen, *Journ. Agr. Sci.* vol. II. p. 109.
[2] Nilsson-Ehle, *Kreuzunguntersuchungen an Hafer und Weizen*, II. p. 76.

plants, each with some four or five leaves, at a time when the rust-epidemic was at its height. Such plants were exposed for the greater part of their lives to the attacks of the parasite and consequently the evidence for their susceptibility or non-susceptibility was as thorough as possible. One disadvantage was not foreseen, namely that these late sown plants would be exposed to the attacks of Black Rust (*Puccinia graminis*). This species when it occurs in this district usually finds the plants at too advanced a stage of growth to make much impression on them. Those showing no signs of ripening, however, are readily attacked. When this occurred in 1907 it became necessary to distinguish between the two species with the result that so much time was lost that the whole series of cultures could not be examined in detail. In order to secure a uniform distribution of uredospores throughout the plots and consequent certainty of chances for infection the cultures were usually arranged so that sowings from immune and susceptible plants alternated with one another. As a rule twenty-four grains from each plant were sown in each row though in a few special cases the number was raised to one hundred. In 1907 the "plant" was partially spoiled by the attacks of field mice but a sufficiently large number of individuals was obtained to give reliable results.

The rust was late in appearing that season (May 10th) and no statistical examination was attempted until June 22nd when it was considered that all the plants which were susceptible would be producing crops of uredospores. At this date the autumn-sown cultures were in full flower whilst the spring-sown rows were "running," that is tillering repeatedly and forming dense masses of foliage from which a scanty crop of ears was being pushed out. Comparisons made between the two series of cultures at this date, and again later, showed that the proportional distribution of the infected plants in each series was the same.

In the following table the results of the examination of the first thirty-six cultures are shown in detail. They may be taken as typical of the 150 examined that year. In the second column the extent of the rust on the $F_2$ plants is noted: "none" signifies that the plant was immune, "medium" that the plant, though badly rusted, was less so than the parent Michigan Bronze, "extreme" that the susceptibility was equal to that of Michigan Bronze. Columns three and four show the number of individuals in each culture which proved to be immune and susceptible.

| Number of Culture | Rust in $F_2$ | Rusty plants in $F_3$ | Immune plants in $F_3$ |
|---|---|---|---|
| 42/5 G  1 | None | 0 | 11 |
| 2 | ,, | 0 | 13 |
| 3 | ,, | 0 | 14 |
| 4 | ,, | 0 | 13 |
| 5 | ,, | 0 | 11 |
| 6 | ,, | 0 | 12 |
| 7 | ,, | 0 | 6 |
| 8 | ,, | 0 | 9 |
| 9 | Medium | 11 | 3 |
| 10 | ,, | 10 | 6 |
| 11 | ,, | 14 | 3 |
| 12 | ,, | 12 | 0 |
| 13 | ,, | 15 | 2 |
| 14 | ,, | 20 | 3 |
| 15 | ,, | 17 | 7 |
| 16 | ,, | 7 | 2 |
| 17 | ,, | 4 | 1 |
| 18 | ,, | 3 | 3 |
| 19 | ,, | 6 | 2 |
| 20 | ,, | 9 | 6 |
| 21 | ,, | 8 | 2 |
| 22 | ,, | 9 | 0 |
| 23 | ,, | 14 | 0 |
| 24 | ,, | 7 | 2 |
| 25 | ,, | 6 | 3 |
| 26 | Extreme | 19 | 0 |
| 27 | ,, | 20 | 0 |
| 28 | ,, | 16 | 4 |
| 29 | ,, | 12 | 0 |
| 30 | ,, | 9 | 1 |
| 42/5 H  1 | Traces | 0 | 57 |
| 2 | Extreme | 36 | 18 |
| 3 | ,, | 47 | 15 |
| 4 | ,, | 38 | 12 |
| 5 | ,, | 30 | 9 |
| 6 | Immune | 0 | 58 |

From it the following facts are evident :—

(1)  That the immune types of the $F_2$ generation breed true to that feature.

(2)  That some of the susceptible forms also breed true but others produce offspring showing segregation into susceptible and immune forms.

(3)  That the "medium class" arc not necessarily heterozygous

(4)  Where segregation occurs the sum total of immune and susceptible individuals is again in the ratio of 1 : 3. The actual numbers for the whole series were 276 and 849.

The results are those that might have been anticipated from those of the $F_2$ generation. They show, conclusively for this particular cross, that the varying degrees of susceptibility are not due to the effects of more than one " dose " of the factor concerned with the production of susceptibility. Many of the immune types were cultivated in the following season and a small number have been grown on until the present (1907—11). These in every case have retained their power of disease resistance completely, though as each culture was in close contact with an excessively susceptible one, so close in fact that the leaves overlapped continuously, they have had every opportunity of becoming infected.

Certain of the homozygous susceptible cultures have also been grown on from 1908 onwards. These were graded as far as possible for their degree of susceptibility and the extent of the rust attack noted and compared in each season. Some have died out completely as the original stock of Michigan Bronze has. The results tend to show that season by season the susceptibility of these cultures as compared with one another is the same. Thus a " medium " culture marked 5 and an extremely susceptible culture marked 10 at the $F_3$ stage earn the same marks in the $F_4$—$F_6$ stages. Slight discrepancies occur in the records but these are undoubtedly due to the difficulty of gauging the difference between say class 5 and 6, or 8 and 9 from season to season.

The varying and probably constant degrees of susceptibility seen in the $F_2$ and succeeding generations, are probably due to the extreme ease with which the degree of susceptibility is altered by slight changes in the plant's metabolism. Any factor altering in any way the metabolic processes of the plant in turn alters the degree to which it is attacked by yellow rust and probably other fungi as well. An extreme case of this is afforded by Salmon's demonstration that the biological form of *Erysiphe graminis* occurring on wheat may attack the foliage of barley which is normally immune to it if appropriate measures are taken. Equally striking examples are provided when the same variety of wheat is treated with various artificial manures. A variety which under ordinary conditions of cultivation would be classed as moderately susceptible may be grown so as to be practically rust-free whilst an adjacent plot of it may in turn be excessively attacked. Without attempting to describe still incomplete investigations on this subject the fact can be illustrated by the incidence of Yellow Rust on the variety Browick grown at Rothamsted in 1910. The intensity of the attack is indicated by the figures in the second column of the annexed

table: 0 indicates almost complete freedom from rust, 12 an excessively bad attack.

| Plot | Rust | Manures | | | | | |
|------|------|---------|---|---|---|---|---|
| 4 | 1—2 | None | | | | | |
| 5 | 2 | Minerals | | | | | |
| 6 | 3 | ,, | +200 lbs. ammonium salts | | | | |
| 7 | 3—4 | ,, | +400 ,, | ,, | ,, | | |
| 8 | 3—4 | ,, | +600 ,, | ,, | ,, | | |
| 9 | 3 | ,, | and nitrate | | | | |
| 10 | 6—7 | Ammonium salts only | | | | | |
| 11 | 10 | ,, | ,, | and superphosphate | | | |
| 12 | 6 | ,, | ,, | ,, | ,, | ,, | and sulphate of soda |
| 13 | 4—5 | ,, | ,, | ,, | ,, | ,, | and sulphate of potash |
| 14 | 12 | ,, | ,, | ,, | ,, | ,, | and sulphate of magnesia |
| 15 | 5 | Minerals + 400 lbs. ammonium salts | | | | | |
| 16 | 9—10 | ,, | +550 lbs. nitrate | | | | |
| 17 | 3—4 | Ammonium salts | alternating each season | | | | |
| 18 | 0 | Minerals | | | | | |
| 19 | 8 | Rape cake | | | | | |
| 20 | 3 | None | | | | | |

The wheat plots at the experimental station of the Royal Agricultural Society at Woburn, where the system of manuring is comparable with those at Rothamsted, showed very similar results though in this case the variety of wheat was Square Head's Master.

Various features can be recognised in the parent plants which affect the plant's metabolism, such for instance as a difference in the leaf area, in the time of maturing, and in the root range of the young plants. These in turn in all probability modify the degree of susceptibility to yellow rust. When segregation occurs fresh combinations of these various features result and consequently new degrees of susceptibility arise.

In the case of the cross American Club × Michigan Bronze the extreme of susceptibility is represented by the latter parent and apparently under our conditions plants more disposed to the attacks of Yellow Rust cannot exist. In many other crosses, however, between immune or slightly susceptible and moderately susceptible varieties, the $F_2$ generation contains individuals far more susceptible to rust than the susceptible parent. A typical case is provided by the cross between Rivet wheat and Red Fife. The former parent can best be described as slightly susceptible. In many seasons it is completely rust free and even in rust years I have never seen the plants carrying more than a few scattered pustules of uredospores. Red Fife has always been more or less badly attacked during the nine seasons it has been under observation. Three $F_2$ generations of this parentage have been raised

containing in all some two thousand plants. Using the parent plants as standards for disease susceptibility the broad outlines of segregation into slightly susceptible and susceptible forms were readily traceable, but amongst the latter group were many individuals far more attacked than even the susceptible parent. Some indeed became so weakened by the attacks of the parasite that they were killed before grain could be produced. The attempts made up to the present to subdivide this group of susceptible plants according to the extent to which they become diseased have not been satisfactory.

One small $F_3$ generation has been raised containing fifty cultures from as many plants chosen from the $F_2$ as representing certain degrees of susceptibility. These were grown in 1911, a year when the rust-epidemic was exceptionally light and when owing to drought many plots failed. It was however evident that in those cultures breeding true to rust susceptibility the most diseased were those descended from especially susceptible $F_2$ plants.

In this particular cross the differences between the parents are unusually numerous and many of them argue differences in the plant's metabolism. There is for instance a great difference in the rate of maturation, Rivet wheat being a very slow growing variety and Fife an exceptionally rapid one for this country. Leaf area and leaf colour also differ markedly, whilst the end product of the metabolism of the two varieties, the endosperm, is so different that one produces the finest the other the poorest type of bread-making flour. In this case some of the differences are recognisable with sufficient certainty to make it possible to continue the investigations with a view of determining how far they control the original degree of susceptibility inherited by the $F_2$ plants.

Whilst none of the cases examined up to the present indicate that susceptibility itself is due to the existence of more than one factor this is undoubtedly not the case where all diseases are concerned. This is illustrated by several crosses in which a disease new to each of the parents has appeared in the $F_2$ generation.

Thus in the past five seasons the occurrence of the sclerotia of *Claviceps purpurea* or ergot has been repeatedly noted in the $F_2$ generations of certain crosses with Rivet wheat. This has been the case in the following crosses:

> Rivet × Red King.
> Sunbrown × Rivet.
> Red Fife × Rivet and its reciprocal.
> Rivet × Galician.

Although confined to crosses with Rivet wheat sclerotia of ergot are not produced in all cases where this variety is used as a parent. For example when crossed with *Triticum polonicum* or with *T. dicoccum* all of the progeny amounting to many thousands of individuals have remained ergot-free.

The Rivet wheat and also the other varieties used as parents have been grown as pure lines for from 7 to 10 years and as each culture has been harvested ear by ear, it is certain that no ergots have occurred on any of them. In fact, apart from these hybrids, no cases of this disease have been seen in any of the cultures grown.

Up to the present the $F_1$ plants, 40 in number, have been ergot-free. The $F_2$ generations have contained on an average 10 per cent. of ergotized plants. The $F_3$ generation raised from plants known to be susceptible has in each case contained some individuals bearing sclerotia but in no case has the number exceeded 25 per cent. of the total number. The $F_3$ generation from plants bearing no sclerotia in $F_2$ has for the most part been ergot-free, but eight cultures out of a total of twenty-four contained a small number of diseased plants.

For the present no significance can be attached to any of these figures for there is no guarantee that every susceptible plant has had an opportunity of becoming infected. Infection in this case appears to occur at the base of the ovary only and this is protected by the paleae throughout the growth of the ear, except at the stage when the flowers open somewhat to shed their empty anthers. The attempt has been made to overcome this difficulty by raising ascophores from the sclerotia and infecting artificially at flowering time but it failed to alter appreciably the percentage of ergotized individuals. This was in all probability due to imperfections in the method, for in several cases ergots appeared on untreated ears of plants where none were produced on treated ears.

The most that can be said, for the present, is that Rivet wheat carries one of the two factors necessary for the production of susceptibility to the attacks of *Claviceps purpurea*. The other factor is to be found in some varieties of *T. vulgare*, but not in *T. polonicum* or *T. dicoccum*. It is probable that *T. vulgare* as a group carries at the most one of these factors only, since a large number of crosses have been made between its varieties without resulting in ergotized individuals.

Further infection experiments are necessary in order to decide what the real ratio of immune to susceptible individuals is before the genetics of this case of disease susceptibility can be unravelled.

Perhaps one of the most important papers published recently with regard to the problem of breeding rust-resistant wheats is that by Pole-Evans[1]. In this he shows that the $F_1$ generation of a cross between varieties respectively resistant and susceptible to the Black Rust (*Puccinia graminis*) is susceptible, and further that uredospores taken from it can in turn infect the resistant parent. The $F_1$ functions in the same manner as Marshall Ward's "bridging species" amongst the Bromes[2].

We have no direct evidence as to whether the same phenomenon occurs with the Yellow Rust. At the suggestion of Marshall Ward, experiments were made on this point in the year 1902 without, however, any definite results being obtained. In consequence they were not continued further. There is sufficient evidence in existence though to show that even if the $F_1$ can function as a "bridge" in this case the effects as far as open field culture is concerned are negligible. Abundant proof of this is found (1) in the fact that the immune parents, most generally employed in these investigations, have remained rust free for eight seasons though grown in close proximity to $F_1$ plants of resistant but susceptible parentage, and (2) in the sharpness of the statistics obtained on the examination of the $F_2$ generations. Such close approximations to the 3 : 1 ratio would be impossible if plants with a constitution RR, similar to that of the immune parent, could be freely infected by the DR plants.

Pole-Evans considers that this fact of "bridging" amongst others renders the breeding of rust-resistant wheats a matter of extreme difficulty. Possibly its seriousness from the economic point of view is over-estimated. Farrer for instance, working under very unsatisfactory conditions, attempted to solve this problem. The success of his efforts may be partially gauged by the fact that Pole-Evans has chosen as a resistant parent one of his hybrid wheats, namely "Bobs."

In the case of breeding for resistance to Yellow Rust experiments have shown that the difficulties are far from insuperable. The one formidable one is to secure resistance in conjunction with all other desirable features. This has necessitated raising many hundreds of cultures, a very small percentage of which have been found suitable for distribution. It is more than probable that the difficulty of combining so many characteristics in one variety has been responsible for the failure of certain rust-resistant varieties to secure permanent places

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[1] Pole-Evans, *Journ. Agr. Sci.* vol. IV. p. 95.
[2] Marshall Ward, *Ann. Mycol.* vol. I. p. 645.

amongst the thousands of wheats in general cultivation. The grower has to lay stress on such important features as yield, time of maturation and quality. He may even be influenced in his choice of varieties by such trifling features as ear-shape. Many examples might be quoted to show that unless all such points are to his liking the variety, though resistant, is doomed to failure. In India, for instance, Yellow Rust causes very serious losses. Many of our English varieties show a considerable degree of resistance under Indian conditions, but if introduced they fail to secure a hold on account of the fact that they mature more slowly than the native varieties. Such facts are I believe a more likely explanation of the failure of resistant types than any supposititious falling off of immunity or gradual advance in the parasitic properties of the rusts. In fact direct evidence that immunity gradually fails is lacking. The oldest hybrid varieties I have any personal knowledge of are now at the $F_8$ stage. They have been under observation continuously both on the University Farm and in general cultivation, and it is safe to say that they are still as resistant as the original $F_2$ plants. Whether they will continue so is a matter for future observation. Still it is perhaps worthy of notice that one of the oldest varieties in cultivation here is Rivet wheat and it is still one of the most resistant of our wheats. Einkorn, possibly the first wheat to be cultivated, is again extremely resistant to the attacks of various rust species. If then historical evidence is worth anything in such matters it goes to show that both the falling-off of immunity and the gradual advance of the parasitic properties of the rusts take place too slowly to influence the work of the plant-breeder.

# STUDIES IN PROTEIN HYDROLYSIS.

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*Preliminary.*

In the following pages the author gives a preliminary account of his attempts to improve the methods commonly in use for the separation of the products of the hydrolysis of proteins.

On surveying these methods, improvements seemed to be possible in the process of esterification. As at present practised the acid liquid resulting from the hydrolysis is first evaporated in vacuo to a syrup. Then alcohol is added and the liquid saturated with dry hydrochloric acid gas. This fails to produce complete esterification on account of the comparatively large amount of water left in the syrup.

The partially esterified acid liquid is again evaporated in vacuo, again diluted with alcohol, and again saturated with hydrochloric acid gas, and this process is repeated twice more.

This tedious process is intended to remove most of the water present in the original syrup and that produced by esterification, but that it fails to do so is evident both from the fact that after separating the esters by Levene's improved method, several repetitions of the whole esterification process are necessary, and from the unsatisfactory yield of amino-acids which may be obtained when all these repetitions have been studiously carried out.

The first attempt to devise an improved method consisted in converting all the amino-acids into lead salts, which after completely drying were digested with excess of diethyl sulphate.

Lead sulphate separated, and esterification took place, but it was found impossible to separate the esters on account of the decomposition of some of the diethyl sulphate into sulphuric acid, which decomposed the esters on distillation.

In the course of these experiments, it was found that the whole of the esters of the various products of hydrolysis, except a small quantity

of diketo-piperazines, were soluble in chloroform, whilst ether dissolved only the esters of the mono-amino acids.

Further experiments were made using dry lead or copper salts with ethyl sulphate, methyl sulphate, ethyl bromide and ethyl iodide, but whilst esterification usually occurred, it was not found possible to devise a practicable method of separating the esters.

During the course of the above experiments, some experience was gained in converting the products of hydrolysis into dry metallic salts, and it seemed possible that one or other of these salts might be utilized for direct esterification with alcohol and dry hydrochloric gas. It was thought that a dry salt might be esterified much more completely than the syrup formerly used which necessarily contains a considerable quantity of water.

The liquid resulting from the hydrolysis of casein by sulphuric acid in the usual way was freed from sulphuric acid by baryta. The barium sulphate was filtered off and carried with it the humin substances always formed in hydrolysis. To the clear light brown liquid excess of litharge or freshly precipitated copper hydroxide was added, and the mixture heated for some time by a current of steam until acidity disappeared. The excess of metallic oxide was filtered off, the liquid evaporated to dryness, and the residue completely dried and powdered.

When hydrolysis had been carried out by means of hydrochloric acid, the acid was removed as far as possible by evaporation on the water-bath and the resulting syrup was then subjected to a current of steam to remove still more of the acid. The liquid was then considerably diluted, heated as before with steam, and precipitated lead hydroxide added until it no longer appeared to dissolve. The humin substances separated as a dark-brown sediment containing much lead, and only a very small amount of nitrogen. When this was removed a light yellow liquid was obtained from which lead salts were obtained as before by means of litharge.

By the above method the copper salts were readily obtained in a dry condition. The final stages in the evaporation and drying of the lead salts are, however, apt to be troublesome.

The following method of esterification has been found to work satisfactorily with both lead and copper salts.

The dry salt was suspended in three times its weight of absolute alcohol, and saturated with dry hydrochloric acid gas at $0°$ C. After standing over night at $0°$ C., most of the hydrochloric acid was removed

by a current of dry air. The liquid was then nearly neutralised with a saturated solution of dry ammonia gas in absolute alcohol, the temperature being kept near 0° C. all the time. It is important that the liquid should remain slightly acid so that the ester-hydrochlorides may not be decomposed. The ammonium chloride which had separated was filtered off, and the alcohol distilled off at low pressure below 40° C. Nearly the whole of the esterification water was shown to be present in the distillate by means of the hydrometer. The remaining syrupy ester-hydrochlorides were dissolved in dry chloroform, in which they are readily soluble, leaving ammonium chloride undissolved.

. The chloroform solution was then shaken with a small quantity of baryta until the latter became granular. This served both to remove the last traces of water and to set free the esters from their hydro-chlorides. The solid residue of baryta, etc. was filtered off, and the chloroform removed as completely as possible from the esters by distillation at low pressure below 40° C. The mono-amino esters were then extracted from the residue by dry ether, in which practically everything readily and completely dissolved, showing that the diamino substances had been retained in the baryta residue.

The filtered ethereal solution was freed from ether and distilled at 15 mm. in a slow current of dry carbon-dioxide gas, the distillation being discontinued as soon as the temperature of the oil-bath in which the distillation flask was heated reached 130° C. By this means decomposition of the esters during the distillation is avoided and the rough isolations of the amino acids described below show that a very good fractionation occurs.

In one experiment from about 300 grams of casein containing 49·6 gm. nitrogen, the residue of esters ready for distillation weighed 241 grams, and on distillation yielded the following results:

| Fraction | Temp. of Bath | Temp. of Vapour | Pressure | Weight of fraction |
|---|---|---|---|---|
| 1 | 90° C. | 45°—50° C. | 25 mm. | 5·04 gms. |
| 2 | 90°—112° C. | 50°—80° C. | 15 mm. | 62·51 gms. |
| 3 | 112°—130° C. | 80°—86° C. | 15 mm. | 11·05 gms. |
| Residue in flask | — | — | — | 94·6 gms. |
| Total ...... | — | — | — | 173·2 gms. |

Fraction 1 was not further examined. From fraction 2, 10·51 gms.

of pure leucine were obtained, and 40·7 gms. of white crystalline acids, presumably the usual mixture of leucine, valine, and proline.

From fraction 3, 1·3 gms. of phenyl-alanine hydrochloride were obtained in the usual way, the remainder resembling the mixed acids of fraction 2. The residue in the flask gave 9 gms. phenyl-alanine hydrochloride recrystallised from hot strong hydrochloric acid, 3 gms. of aspartic acid which was separated as barium aspartate, and 24·4 gms. of glutaminic acid hydrochloride has already been separated. The experiment described has been advanced to this stage in under three weeks' work.

The author claims that by using dry salts for the esterification in the place of the watery syrup used heretofore, a very great saving of time and trouble is effected in the early stages of the process, and that a very satisfactory esterification is obtained, as the presence of much alcohol prevents the reverse effect of the small amount of water formed. The complete removal of water from a syrup is prevented by the presence of much free hydrochloric acid, and the removal of the esterification water is therefore more efficiently secured when, as in the author's process, an alcoholic solution containing the ester-hydro-chlorides alone is evaporated.

It is further claimed that owing to the solubility of the ester-hydro-chlorides in chloroform and the freedom of their solution from hydro-chloric acid, the anhydrous baryta in small quantity and without the addition of water efficiently liberates the esters from their hydro-chlorides.

The yields are as good as those obtained by the older methods. The author is pursuing the investigation and is already conscious of several important details in which improvements can be effected.